Photo-activation therapy using thallium

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Introduction

The feasibility of photo-activation therapy using synchrotron radiation in combination with high-Z contrast agents has earlier been demonstrated by others. Recently, at the European Synchrotron Radiation Facility (ESRF), experimental treatments of F98 glioma bearing rats using iodine as contrast agent (Iomeron) have shown promising results [1,2]. However, as Iomeron remains extracellularly, the effect of the short-ranged Auger-electrons following the photo-reactions in iodine cannot be fully utilized. By using cis-platinum as contrast agent, however, experiments in the same rat glioma model have resulted in extremely prolonged survival times. In one study, 34% of the treated animals were still alive after 1 year [3]. It was first suggested that this large effect was due to DNA-damage from radiation-induced Auger-electron emissions from internalized cis-platinum [4] but later, it was explained as a combined effect of chemo-therapy and radiation [5]. Further studies on the importance of Auger electron from agents used with photo-activation therapy are therefore warranted. Another agent with well documented intracellular uptake is thallium. Radioactive thallium (^{201}Tl) has earlier been commonly used for diagnostic purposes in nuclear medicine for heart and gliomas. It has also been used by our group for experimental treatment of rat glioma [6,7]. After intratumoural injections of small amounts of radioactive thallium (3x5 MBq) a high therapeutic effect was demonstrated (7). This was believed to be due to an intracellular uptake of thallium via the Na-K-ATPase pump activity, possibly even into the cell nucleus, leading to the emission of numerous low-energy Auger electrons in the very close vicinity to the chromosomes and the DNA. The mean absorbed dose calculated based on the MIRD scheme can not explain this high therapeutic effect. With this background, it is of great interest to investigate the potential of thallium as an agent in photo-activation therapy. In this work, we investigate the hypothesis that the therapeutic effect of monochromatic synchrotron radiation may be enhanced by Auger electrons emitted due to photo-stimulation of pre-administered thallium.

Materials and Methods

Animal model

In this study, we have used Fischer-344 rats together with the RG2 glioma cell-line. The RG-2 rat glioma has a highly invasive growth pattern, similar to human glioblastoma multiforme (8). The rats were inoculated in our own laboratory at Lund University by injecting 5000 RG2 cells in 5 µl nutrient solution with a Hamilton syringe into the head of the right caudate nucleus. To avoid extracranial tumour growth, the injection site was cleaned with 70% ethanol after injection and the borehole sealed with wax. Four days after the inoculation, the rats were transported by air to the ESRF facilty in Grenoble, France. All experimental animal procedures were approved by the local Animal Ethical Committee (#M50-10).

Treatment groups

A total number of 46 rats were divided into 5 different treatment groups as shown in Table 1. The animals were anesthetized during all treatments by using ketamine/xylazine. For groups A-C, radiation treatments at ESRF began on day 7 after the inoculation. One hour prior to the radiation treatment, the rats in groups A and B received an intratumoral injection of stable Tl; ^{203}Tl (75 µg ^{203}Tl in a 5 µl volume). This dose level was determined after previous preparatory cellular uptake studies and toxicity tests in rats (data not shown).

Group	*No of rats*	*Treatment*
A	10	Thallium injection + irradiation with energy above the K-edge of Tl
B	10	Thallium injection + irradiation with energy below the K-edge of Tl
C	8	Irradiation with energy above the K-edge of Tl
D	8	Thallium injection
E	8	No treatment

Table 1. The subdivision of animals into the different treatment groups.

Irradiation

The irradiations were performed at the experimental station of the ID17 beam-line at the ESRF. After careful set-up in a stereotactic frame, see Figure 1, the rats were irradiated with a 10x2 mm^2 rectangular beam while they were rotated in order to deliver a homogeneous photon fluence throughout the entire tumour volume. After one completed rotation, the frame was moved 2 mm vertically, and the irradiation was continued. This was repeated five times in order to cover 10 mm in the cranio-caudal direction. The energy of the impinging photons was mono-energetic with 300 eV above or below the K-edge of thallium (85.5 keV). The treatment was given in one fraction of 10 Gy.

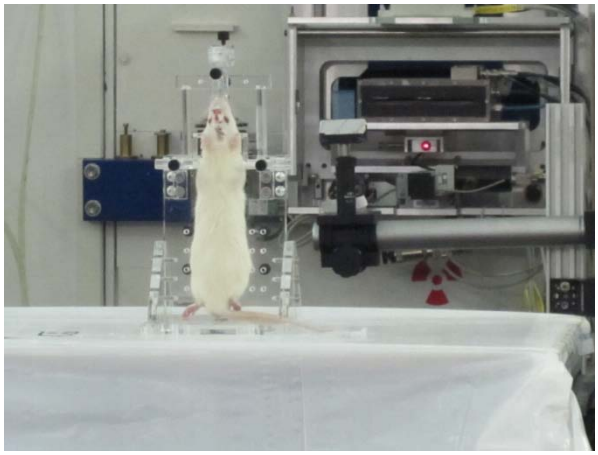

Figure 1. The animal irradiation set-up at the ID-17 beam-line.

Dosimetry

Careful dosimetric measurements were made in order to confirm the absorbed dose level in each animal. The beam current was continuously monitored and used as reference by assuming a constant photon fluence per beam current. Dosimetric measurements were done with 3 cm solid water, and an ionization chamber (UNIDOS; PTW-Freiburg) placed at a depth of 1.5 cm. Based on the measurements, the dose rate was calculated as the absorbed dose per second and per beam current.

The absorbed dose to the tumour was calculated as follows:

$$D_{tumour} = \dot{D} \cdot I \cdot t \cdot k_{field\ size} \cdot k_{scatter},$$

where $\dot{D}$ is the dose rate per unit beam current, I is the beam current, t is the irradiation time and $k_{field\ size}$ and $k_{scatter}$ are correction factors that account for the change from reference setup to treatment setup. With all factors known, the irradiation time for each rat was calculated by rearranging the equation above:

$$t = \frac{D_{tumour}}{\dot{D} \cdot I \cdot t \cdot k_{field\ size} \cdot k_{scatter}}$$

Imaging

For all the irradiated rats, synchrotron-CT imaging was performed at 300 eV above the K-edge. One example is displayed in Figure 2, where soft tissue is shown in blue color, and skeleton parts are white. A high Tl-uptake in the tumour is shown in yellow-red. One additional rat was used for high-resolution imaging with the aim to study the bio-distribution of thallium on a sub-mm level.

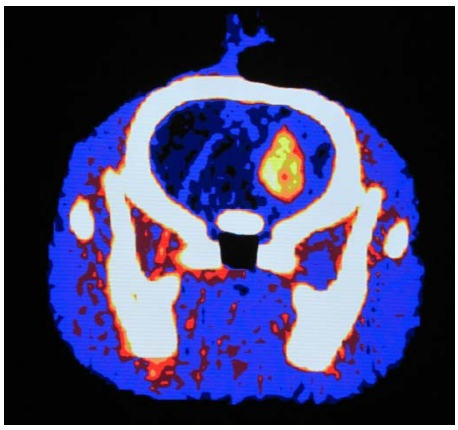

Figure 2. An example CT-image, demonstrating the high Tl-uptake in the tumour.

Follow up

At the end of the experiment, all surviving rats were transported back to our laboratory at Lund University for continued follow-up. The effect of the treatment was evaluated in terms of survival time. The animals were observed daily for symptoms of tumor growth, such as keeping their heads turned to one side, rotating or losing weight. Other signs include unwillingness to move, shaggy fur and reddening of eyes and nose. When an animal developed such symptoms, it was euthanized and the brain was excised and put in formalin. Two rats were excluded from the analysis due to illness.

Results

The survival data for the different treatment groups are presented in Figure 3, and the average survival times for the rats in the different treatment groups are listed in Table 2.

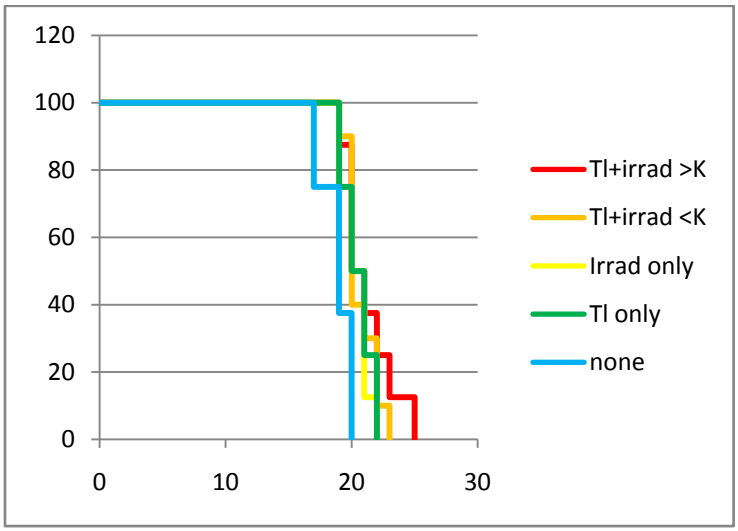

Figure 3. Survival curves for the different treatment groups.

Group	*Average survival*
A	21.3
B	20.7
C	20.4
D	20.5
E	18.9

Table 2. Average survival time for the different treatment groups.

The difference in survival time between the different groups was tested for significance using a log-rank test for the Kaplan-Meier survival functions. At a significance level of p=0.05, all treated groups showed an increased survival time as compared to the untreated group (E). There was, however, no statistically significant difference between the treated groups. The same results were obtained by using Mann-Whitney or t-test.

Discussion

In this work, we have investigated the hypothesis that the therapeutic effect of monochromatic synchrotron radiation may be enhanced by the emission of Auger- and other low-energy radiation following photo-absorption in pre-administered thallium. As a test model we used RG2-glioma bearing Fischer-344 rats, divided into several groups receiving different treatments according to Table 1.

It was first established that synchrotron radiation has a therapeutic effect by itself, albeit rather small at the given dose level. The average survival time for all groups treated with radiation (A – C) was longer (p=0.05) than for the untreated group (E), see Table 2.

If the hypothesis was valid, it was therefore expected that the therapeutic effect should be even more pronounced in group A than in group B, since the probability for photo-absorption is far greater

at an x-ray energy just above than slightly below the K-edge of thallium. According to our results, however, there was no statistically significant difference in survival time between the two groups.

We believe this failure may be due to the microscopic bio-distribution of the thallium. It is known from earlier work that thallium is incorporated intracellularly via the Na-K-ATPase pump activity, possibly even into the cell nucleus. Because of the extremely short range of the Auger- and other low-energy electrons, however, a successful outcome of this regime would require an accumulation of thallium in the very close vicinity to the chromosomes and the DNA. Our results suggest that this is not the case. The therapeutic results obtained in our previous work with radioactive thallium may then have to be re-interpreted as being a consequence of the additional internal conversion electrons with higher energy and a longer range.

In our future experiment, we will therefore aim at producing also photo-electrons with a range long enough to cross a cell-diameter. With thallium, this means that we will need to increase the x-ray energy from 85.8 keV, as was used in this study, to about 100 keV (the optimal energy will be determined by preparatory Monte Carlo calculations). At this higher energy, the dose enhancement is only slightly lower than just above the K-edge. With a reasonable estimate of the attainable thallium concentration, a dose enhancement factor of about 1.5 could be expected. As the relative biological effect of the photo-electrons may not be as large as expected for the Auger-electrons, we will therefore also increase the x-ray dose from 10 Gy to 15 Gy.

At this stage, all data have not yet been analyzed. In addition to the survival data presented here, we will also investigate the histological and morphological effects in the brain and possible tumour rests by immunohistochemical assays. We will also characterize tumour cells from the tumour samples with flow cytometric analysis, with regards to DNA- and S-phase ploidy. Furthermore, we will use test object images acquired at the beginning of the experiments to derive fundamental parameters for thallium imaging. Finally, we have collected a large number of *in vivo* images during these experiments, which we expect contain interesting information about the uptake and bio-distribution of the injected thallium.

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