Continued trials on photo-activation therapy using thallium

C Ceberg[1], B-A Jönsson[1], G Grafström[1], T Larsson[1], K Wingårdh[1], S Strömblad[2], H Nittby[2], Y Prezado[3], H Elleaume[4], LG Salford[2], B Baldetorp[5], S-E Strand[1]

Departments of [1]Medical Radiation Physics, [2]Neurosurgery, and [5]Oncology, Lund University, Sweden, [3]Biomedical Beamline ID17, ESRF, and [4]Institut des Neurosciences, Grenoble, France

Introduction

Photo-activation radiotherapy (PAT) relies on the administration of a drug containing a high-Z element prior to external irradiation with monoenergetic X-rays (see *e.g.* Bencokova *et al.* 2008). Photo-interactions between this drug and the impinging synchrotron radiation, tuned to a proper energy level matched to the absorption cross-section of the administered element, will produce low-energy photo-electrons and Auger electrons resulting in a high local dose. If the drug is taken up preferentially by the tumour cells, this method can potentially yield a very large therapeutic dose ratio between tumour and normal healthy tissue. Pre-clinical trials of PAT have previously been carried out on glioma carrying rats with promising results using different tumour targeting agents containing high-Z elements, such as iodine (Adam *et al.* 2006) and platinum (Rousseau *et al.* 2007). Other elements have also been suggested, such as gadolinium (De Stasio *et al.* 2006) and gold (Hainfeld *et al.* 2010).

The purpose of this project was to investigate the therapeutic potential of PAT in the RG2 rat glioma model using thallium as tumour targeting agent. The uptake of thallium in living cells has been studied extensively and is well documented. With particular relevance for this work, it has been shown that cultured human glioma cells have a high specific thallium uptake, which is mediated by similar transport mechanisms as the potassium uptake (Brismar *et al.* 1995a). It has been conjectured that an elevated thallium uptake may be related to changes in pH (Brismar *et al.* 1995b). For the RG2 rat glioma cells, we have demonstrated a high cellular uptake in our own laboratory. Investigations of the intracellular thallium distribution have shown that it mainly stays in the cytosol, both in cultures of rat myocardial cells (Arbab *et al.* 1998) and in hepatic and renal cells extracted from rats given intra-peritoneal injections of thallium (Sabbioni 1980). In another study on cells from tumour-bearing mice, however, a small proportion of the administered thallium was found to be localized and bound to protein in the nuclear, mitochondrial and microsomal fractions (Ando *et al.* 1987). When administrated *in vivo*, thallium accumulates predominantly in viable tumour tissue, less in normal or inflammatory tissue, and least in necrotic or non-active tissue (Fukumoto 2004). These properties have been utilized for a long time in clinical medicine, and the diagnostic possibilities of thallium (^{201}Tl) scintigraphy in cerebral lesions of various kinds were described already in the 1970's (Ancri *et al.* 1978). Normally, there is little or no uptake in the white matter of the brain. Brain tumours, however, in particular malignant gliomas, often exhibit an intense uptake of thallium with a strong tendency of prolonged retention (Kjällen *et al.* 1996). For the RG2 rat glioma model used in the present work, we have previously investigated the intracerebral biodistribution of radioactive thallium (^{201}Tl) after intra-tumoural administration by using digital autoradiography (Ljunggren *et al.* 2004). Correlation with histology confirmed a very low activity concentration in the normal brain

tissue, whereas a clear uptake could be demonstrated both in the primary tumour at the injection site and in metastases distant from the injection site (Ljunggren *et al.* 2004).

With this background, we believe it is of great interest to investigate the potential of stable thallium (^{203}Tl) as a targeting agent for PAT. Hitherto, two experimental sessions have been conducted at the ID17 beamline of the ESRF; MD-484 in May 2010 and MD-563 in February 2011. Related studies not requiring synchrotron radiation have been carried out in parallel at our home department.

Materials and Methods

Animal model

In these studies, Fischer-344 rats were used together with the RG2 glioma cell-line. The animals were ordered directly to the Biomedical Facility at the ESRF and kept there during the entire duration of the experiments and the follow-up time. The cells were inoculated into the head of the right caudate nucleus, where a tumour developed with fatal outcome within about 3 weeks if the animal was left untreated. The RG2 rat glioma is very aggressive (as compared, for instance, with F98) and has a highly invasive growth pattern, similar to human glioblastoma multiforme (Barth and Kaur 2009).

Uptake and biodistribution of thallium

A tumour cell specific thallium uptake is essential for the success of PAT, and uptake and biodistribution studies are therefore of great importance for this project. During the irradiations at the ESRF we performed element-specific imaging of the distribution of the high-Z agent in the living rat brain by quantitative K-edge subtraction CT. Preliminary results from such studies are shown below. In addition, we plan to conduct parallel investigations with radioactive thallium (^{201}Tl) by using micro-SPECT (resolution 0.5 mm) at the Lund University Bioimaging Center (LBIC), as well as digital autoradiography with a spatial resolution of 75 μm. These studies will also be complemented with microscopic studies by using the Lund Nuclear Microprobe, which will add important information for the interpretation of the results of the pre-clinical PAT trials.

Theoretical dosimetric calculations

PAT experiments pose significant dosimetric challenges. An important part of our program was therefore to address the issues of reference dosimetry, correction factors for treatment conditions, and *in vivo* dosimetry. This was done both by using measurements and Monte Carlo calculations with the Penelope software system. Monte Carlo simulations were also used to estimate the dose enhancement factor and the therapeutic gain that can possibly be obtained with PAT.

Pre-clinical trials of PAT

The PAT treatments were performed at the existing biomedical station of the ID17 beam-line at ESRF, which fulfills the necessary requirements for the experiment (white or monochromatic beams from 20 to 100 keV). All animal experiments, including the transports to the ESRF, were carried out with approval from the local ethical committee (current approval #M50-10).

The treatments took place at day 8-10 after the inoculation, and 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, see Figure 1.

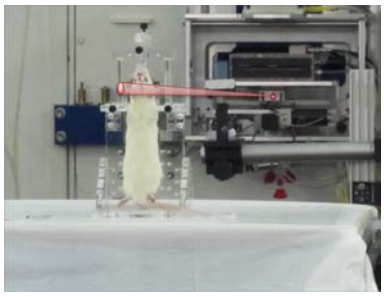

Figure 1. *A tumour carrying rat in the PAT treatment position in the ID17 beamline at the ESRF.*

The energy of the impinging photons was monoenergetic with an energy of either 50 keV, or just above the K-edge of thallium (85.5 keV), as discussed below. One control group was also treated at an energy just below the K-edge. The treatment was given in one fraction of 10 Gy or 15 Gy. One hour prior to the radiation treatment, the rats received an intratumoral injection of ^{203}Tl (75 μg ^{203}Tl in a 5 μl volume). At the end of the experiment, all tissue samples were brought back to our laboratory at Lund University for continued follow up. The effect of the treatment was primarily evaluated in terms of survival time. By immunohistochemical assays we also plan to study histological and morphological effects in the brain and possible tumour rests, and tumour cells from the frozen samples will be characterized with flow cytometric analysis, including DNA ploidy and S-phase (work-in-progress).

Results

Uptake and biodistribution of thallium

At the time of the PAT experiments, we performed quantitative element-specific synchrotron CT, see Figure 2. It was then found that the thallium concentration after intra-tumoural injection was around 2-5 μg/g in the region of the tumour.

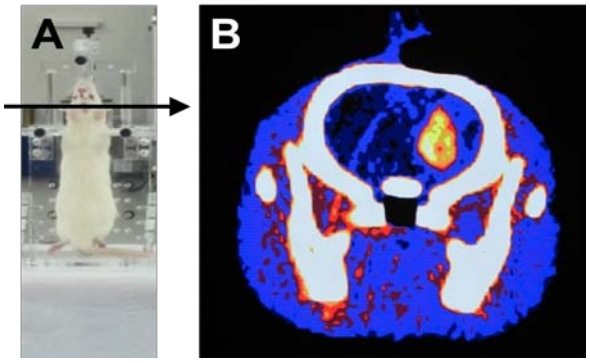

Figure 2. *A) Experimental set-up for PAT in the ID17 beamline at the ESRF. B) Synchrotron CT image from a slice through the tumour volume showing the thallium uptake in yellow.*

For the comparison of the Tl-distribution with histological preparations, high-resolution synchrotron CT images are required. Preliminary results from this work-in-progress are shown in Figure 3.

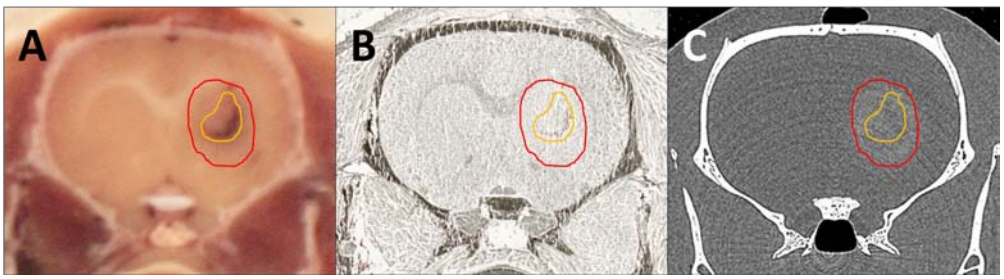

Figure 3. *A) A photographic reproduction of a transversal section through the tumour. B) Histological preparation of the same slice (unstained). C) High-resolution synchrotron CT of the same slice showing the thallium distribution. The thallium uptake is well correlated to the peripheral growth of the tumour (red contour), while the central necrotic area show less thallium uptake (orange contour).*

Theoretical dosimetric calculations

Theoretical calculations on the dose enhancement effect of thallium were carried out. According to these calculations, we decided to investigate the effect of Tl-enhanced PAT at two different synchrotron radiation energies; 50 keV and 86 keV (the latter just above the K-edge of thallium). While the background absorbed dose without thallium was kept constant at either 10 Gy or 15 Gy, an additional contribution originated from interactions with thallium. At the higher energy (86 keV), the secondary K-electrons had a very short range, resulting in extremely high specific energy deposition close to the interaction site. If the thallium accumulated close to the sensitive cell nucleus, these energy depositions could be radiobiologically very effective. At the lower energy (50 keV), the secondary L-electrons produced in the photo-reactions had sufficient energy to reach several cell diameters, which would result in a more homogeneous exposure to the cell and its different components. In this case, the radiobiological effect was not expected to differ significantly from that of external irradiation. On the other hand, at this energy the absorbed dose enhancement factor was according to our calculations at its maximum, see Figure 4. A manuscript reporting on these results is under preparation ("Pre-clinical dosimetry for thallium-enhanced synchrotron radiotherapy").

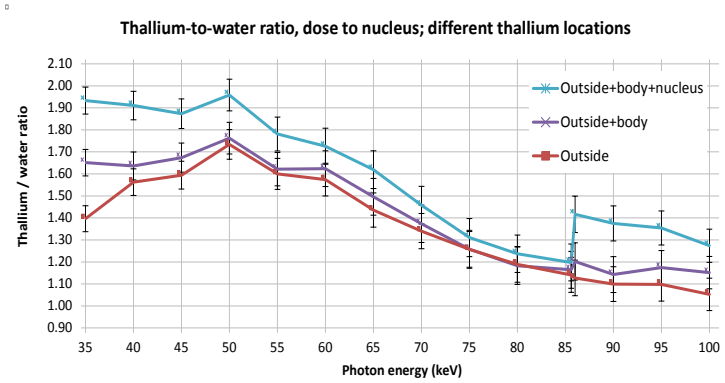

Figure 4. *The dose-enhancement factor of thallium, scored in a small volume representing the cell nucleus, as a function of synchrotron radiation energy. The three curves assume that thallium is located only outside the cells (red curve), outside the cell nucleus (purple), or evenly distributed (blue).*

Pre-clinical trials of PAT

In our initial experiments we used thallium enhanced PAT for rats carrying RG2 gliomas at two different dose levels, 10 Gy and 15 Gy (thallium contribution not included). The synchrotron radiation energy was either 50 keV or just below or above the K-edge of thallium. Six to nine animals were included in each treatment group. The resulting survival curves are shown in Figure 5, and the median survival times are presented in Table 1.

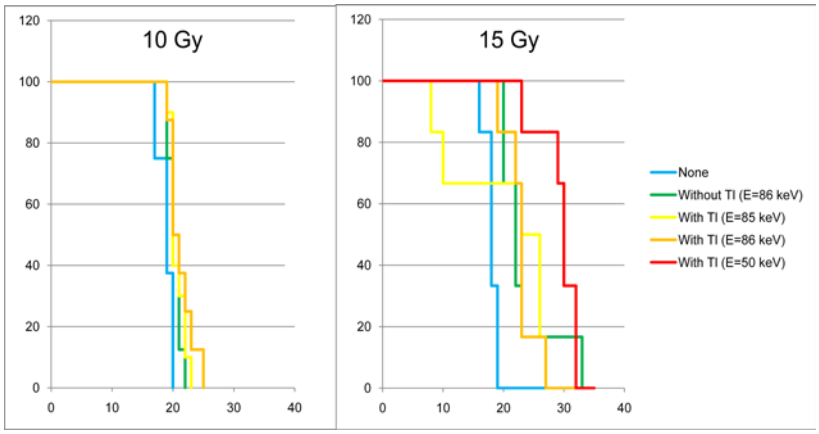

Figure 5. *Survival curves (percentage of the animals alive as a function of days after inoculation) for the RG2 glioma carrying rats treated in our initial experiments.*

Treatment	10 Gy	15 Gy
None	19	18
Without Tl (E=86 keV)	20.5	22
With Tl (E=85 keV)	20	24.5
With Tl (E=86 keV)	20.5	23
With Tl (E=50 keV)	-	30

Table 1. *Median survival times in days for the RG2 glioma carrying rats after synchrotron radiation therapy at 10 Gy or 15 Gy, with or without thallium. Untreated control animals are included for comparison.*

The survival data was analyzed using the log-rank test based on the Kaplan-Meier survival estimate, and statistical significance was defined as $p<0.01$. For the animals irradiated to 10 Gy there was no statistically significant difference, neither between the groups treated with or without thallium, nor between the groups treated with the energy below or above the K-edge. This was also the case for the animals irradiated to 15 Gy. For the animals irradiated without thallium, however, a slightly increased median survival time was observed as a function of absorbed dose. The prolonged survival of the animals irradiated to 15 Gy at 50 keV was statistically significant as compared to all other treatment groups.

Discussion

In the beginning of the 1980's, it was observed both in cell cultures and *in vivo* in mouse testes, that ^{201}Tl, with its EC decay, was much more toxic than expected (Kassis *et al.* 1983, Rao *et al.* 1983), in particular compared to the similarly distributed beta emitter ^{204}Tl (Rao *et al.* 1983). We have made similar observations in the RG2 rat glioma model, for which we have previously demonstrated an unexpectedly high therapeutic effect of intratumoural injections of small amounts of ^{201}Tl (Sjöholm *et al.* 1995), that could not be explained by the low mean absorbed dose calculated based on the MIRD scheme (Stabin *et al.* 2008). Consequently, this was believed to be due to an intracellular uptake of thallium, possibly even into the cell nucleus, leading to the emission of radiobiologically effective low-energy Auger electrons.

From the results of the present study, a weak dose-response relation was observed for the animals treated with radiation only. An improved therapeutic effect was also noted due to thallium accumulated in the tumour tissue, as the dose-enhancement at 50 keV had a significant effect on the median survival time. However, an enhanced radiobiological effect of thallium entering the cell nucleus could not be observed in these studies, since the effect of radiation at around 86 keV was not significantly affected by i) if thallium was used or not, or ii) if the energy was below or above the K-edge, see Table 1. Thus, the experimental results at the selected energies provided very important data for the interpretation of the radiobiological effects of thallium. A manuscript reporting on these results is under preparation ("Prolonged survival of RG2-glioma bearing Fischer rats after thallium-enhanced synchrotron radiotherapy").

Although we were not able to cure any of the glioma bearing rats in these initial trials, the survival time was increased by nearly 70% for the rats treated at 50 keV. These results are to be viewed as preliminary, and more data is needed. Once the present findings are confirmed, attempts will be made in order to further improve the situation, for instance by optimizing the treatment parameters, or by using a combination of PAT with immunotherapy.

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