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Necrosis of malignant gliomas after intratumoral injection of ^{201}Tl *in vivo* in the rat

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Fourteen adult Fischer 344 rats were inoculated *in vivo* unilaterally in the caudate nucleus in the brain with malignant RG 2 glioma cells. By 3 weeks a tumor with a diameter of 3–6 mm normally develops. Ten animals which survived the repeated periods of anesthesia and thallium (Tl) injections (intratumorally three times of ^{201}Tl, 15–23 days after inoculation) showed a prolonged retention of radioactivity at the site of injection with no uptake in other organs except for the kidneys. Singular circumscribed necroses were found post-mortem at the site of injection, comprising malignant glioma tumor tissue, which in six animals was absent, in three animals was markedly reduced in size compared with controls and in one animal had the expected size. In four animals metastases were found in distant locations in the brain; in three of these cases there was a retention of radioactivity in the tumor. The selective necrotizing effect on the tumor cells is interpreted as mainly due to emission of Auger electrons from intracellularly accumulated ^{201}Tl, giving rise to very high energy deposition in the vicinity of the cell nucleus. The results should also have implications for the treatment of human malignant gliomas.

Key words: Auger electrons; β-camera, brain tumor, gliomas, thallium.

Introduction

External radiation therapy has only a marginal effect on human malignant gliomas as regards inhibition of tumor growth and survival time of the patient.[1] The limitation is unacceptable side-effects if the absorbed dose is increased beyond what is generally approved in the clinical context at present. However, the insensitivity of the glioma cells to radiation is presumably only relative. If the radiation energy imparted intracellularly or in the vicinity of the glioma cells could be increased, it is likely that these cells could be forced to necrotize.

^{201}Tl is a radioactive isotope with a half-life of 72 h which accumulates selectively in malignant gliomas *in vivo*.[2–6] This feature is used in non-invasive neuro-imaging diagnosis of malignant gliomas with ^{201}Tl and SPECT.[2–6] Brismar *et al.*[7] have shown that the permeability for potassium in the cell membrane of malignant glioma cells *in vitro* is increased and that ^{201}Tl can be regarded as a potassium analogue. This is most likely the pathophysiological mechanism for the selective uptake of ^{201}Tl in malignant gliomas *in vivo*.

The object of the present study was to investigate if locally deposited ^{201}Tl in malignant gliomas *in vivo* in rats could have a deleterious effect on the glioma without affecting the surrounding normal brain tissue. Fischer 344 rats with RG 2 malignant glioma cells inoculated in the brain were used, and the distribution of radioactivity and effect on the cellular level were assessed with a newly developed β-camera[8] and histopathological examination post-mortem.

The concept of intratumoral injection of radiopharmaceuticals for therapy has been used in earlier studies. For example, both labeled monoclonal antibodies[9] and ^{32}P[10] have been injected in glioma patients. Our present study, however, is the first one where only radionuclide ions have been injected intralesionally, without labeling to any tumor seeking agent or utilizing particles trapped in the tissue.

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Materials and methods

Fourteen adult female Fischer 344 rats aged 12 weeks and with a mean weight of 250 g were used. The animals were anesthetized with chloralhydrate i.p. before they were mounted in a stereo-

tactic frame as previously described.[11,12] Using a small burrhole in the skull, the tip of a needle connected to a Hamilton syringe was placed stereotactically unilaterally in the head of the caudate nucleus. A suspension of around 5000 *in vitro* cultured malignant RG 2 glioma cells[13] in 5 ml nutrient solution was injected slowly through the needle. The scalp was then sutured.

Eleven of the 14 rats were administered 5 MBq ^{201}Tl intratumorally on days 15, 17 and 19 after inoculation. The anesthesia, stereotactic procedure and target for the injection were the same as during the inoculation of cells at the start of the experiment. ^{201}Tl chloride was dissolved in 10 µl saline and it was injected manually slowly during 10 min in order to avoid local overflow. The scalp was sutured after each injection.

One hour after the first ^{201}Tl injection the whole bodies of two animals were imaged with a scintillation camera in order to measure the distribution of radioactivity. The same imaging was repeated the following day, i.e. 1 day before the second ^{201}Tl injection.

On day 21 after inoculation the animals were sacrificed with an overdose of chloralhydrate i.p. The skull was quickly opened and the intact brain was removed, frozen in liquid isopenthane cooled in liquid nitrogen and stored at −70°C until further processing.

Three of the 14 rats were administered 5 MBq ^{201}Tl on days 15, 19 and 23 after inoculation, and were sacrificed 4 days after the last injection. The procedure was otherwise identical to that described above except that no whole body measurements in the γ-camera were performed in this group.

The same day as the brain was removed from the animal it was freeze-sectioned in a microtome in parallel slices 20 µm thick. One slice through the middle of the tumor was placed on a perspex glass and transferred to a recently developed β-camera[8] for measurements of the distribution of radioactivity in the slice. The camera consists of a thin plastic scintillator and a light sensitive micro-channel plate detector. The tissue sample is mounted on the scintillator with a plastic film. The system is housed in a freezer and the measurements can be performed at −20°C. Details of the camera can be found elsewhere.[8]

Adjacent slices to the one which was measured in the β-camera were stained with hematoxylin & eosin and examined in a light microscope for histopathological classification.

As a control group we used Fischer 344 rats that had been inoculated with RG 2 malignant glioma cells in recent years in various projects.[11,12,14] This group comprises about 2000 animals. The rate of tumor growth after inoculation exceeds 99% and the tumor diameter 21 days after inoculation is 3–6 mm, leading to death of the animals 21–24 days after inoculation (Figure 1).

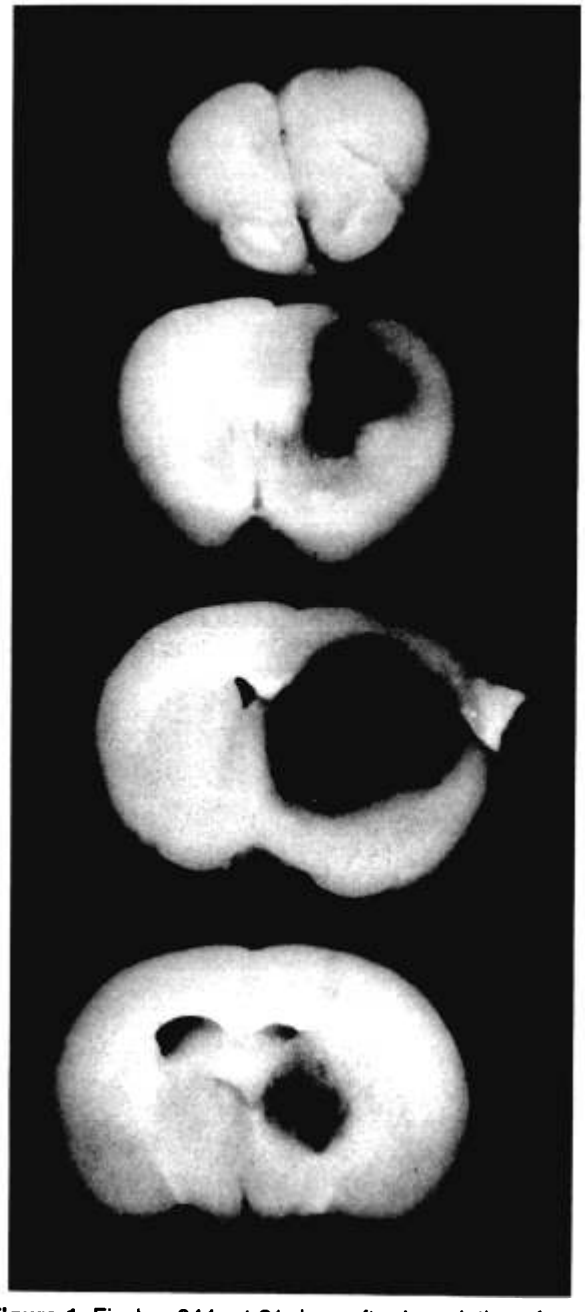

Figure 1. Fischer 344 rat 21 days after inoculation of malignant RG 2 glioma cells in the caudate nucleus in the brain. Coronal sections showing extent of the tumor as dark color (cresyl violet staining).

Results

Three animals from the group of 11 rats that received ^{201}Tl injections at 2 day intervals did not survive the repeated periods of anesthesia and were excluded.

The whole body scintillation camera images of two rats at 1 h after the first intratumoral injection of ^{201}Tl showed that almost all radioactivity was retained in the brain (Figure 2, left). Due to the spatial resolution of the camera, one cannot discriminate between tumor and normal tissue. The repeated imaging on the following day without any new injection of ^{201}Tl showed that a large part of the radioactivity was still retained in the brain, but also that there was some activity in the kidneys (Figure 2, right). The kidneys from one animal were sectioned post-mortem and examined histopathologically. No signs of tissue damage could be detected. Examination with the β-camera revealed that most of the radioactivity was located in the collecting tubules.

The eight rats that received three ^{201}Tl injections intratumorally, with 2 day intervals, were examined histopathologically. They all showed one single circumscribed necrosis with a diameter of 2–4 mm located in and close to the caudate nucleus (Figure 3, left column). There were no signs of any tissue damage in the normal tissue surrounding the necrosis or in the contralateral hemisphere. In six rats there was no viable tumor tissue within or adjacent to the necrotic area (Table 1 and Figure 3, upper row). In one rat there was a small area of viable tumor tissue at the edge of the necrosis making up not more than 10% of the total necrosis–tumor area (Figure 3, middle row). In another rat there was viable tumor tissue within the necrotic area occupying about 50% of the total necrosis–tumor area. In four rats tumor tissue was found at distant locations, well separated from the necrotic area, either superficially in the injection canal or at the base of the brain (Figure 3, bottom row). There were no signs of necrosis in any of these distant tumors.

The β-camera measurements showed that areas with high radioactivity coincided with active tumor tissue and that necrotic areas corresponded to low radioactivity (Figure 3, upper and middle rows). Focal accumulation of radioactivity in tumor tissue at a distance from the original tumor site, which was totally or partially necrotic, was demonstrated in three of the four animals with distant tumors (Figure 3, bottom row). No radioactivity was found in normal tissue in any of the animals.

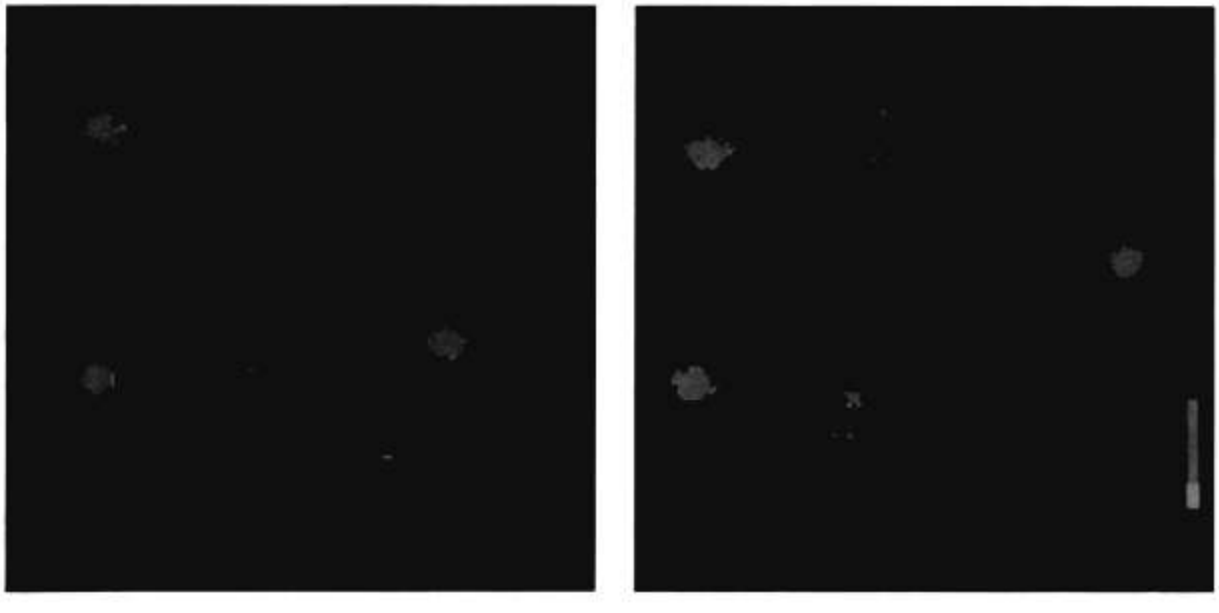

Figure 2. Whole body imaging with a scintillation camera of two Fischer 344 rats with malignant RG 2 glioma cells inoculated 15 days earlier, 1 h after injection of 5 MBq ^{201}Tl intratumorally in the caudate nucleus in the brain (left). Note focal accumulation of radioactivity in the brain of the animals. A test tube, also with 5 MBq ^{201}Tl, is placed outside the animals as a reference. Whole body imaging with a scintillation camera of the same animals 24 h later without any new ^{201}Tl injection is shown on the right. There was still a high accumulation of radioactivity in the brain of the animals corresponding to the activity level in the test tube which had been subjected to the same isotope decay as the activity in the body. Note increased activity in the kidneys. Yellow color denotes high activity level.

Table 1. Relative distribution of necrosis and remaining tumor post-mortem at the injection site of ^{201}Tl in the caudate nucleus in the brain of eight rats that received ^{201}Tl injections on days 15, 17 and 19 after inoculation of malignant RG 2 glioma cells

Rat	Injection site		Tumor in other locations
	necrosis (%)	tumor (%)	
1	100	0	
2	100	0	
3	100	0	
4	90	10	
5	50	50	inj canal + basal
6	100	0	basal
7	100	0	inj canal
8	100	0	inj canal

Among the three rats that received ^{201}Tl at 4 day intervals, two animals survived the anesthesia periods. One rat had a small tumor at the edge of the necrosis, whereas the other rat had developed a tumor comparable in size to untreated controls. When these rats were sacrificed on day 27 after inoculation they had survived longer than the mean of the controls (22.5 days).

Discussion

Although the present sample is small, the results raise some interesting points. ^{201}Tl injected daily into the tumor is most likely taken up by the glioma cells. The evidence for this is the following. It is known from *in vitro* experiments that there is a preferential uptake of ^{201}Tl in malignant glioma cells compared with normal glia cells or neurons.[7] There was a prolonged retention of ^{201}Tl in the brain with no focal uptake in other organs except for the kidneys, where the radioactivity was concentrated in the collecting tubules. This indicates uptake in an intracellular brain compartment that favors influx of ^{201}Tl as is the case for malignant glioma cells. It is likely that elimination from the extracellular compartment was much faster via the

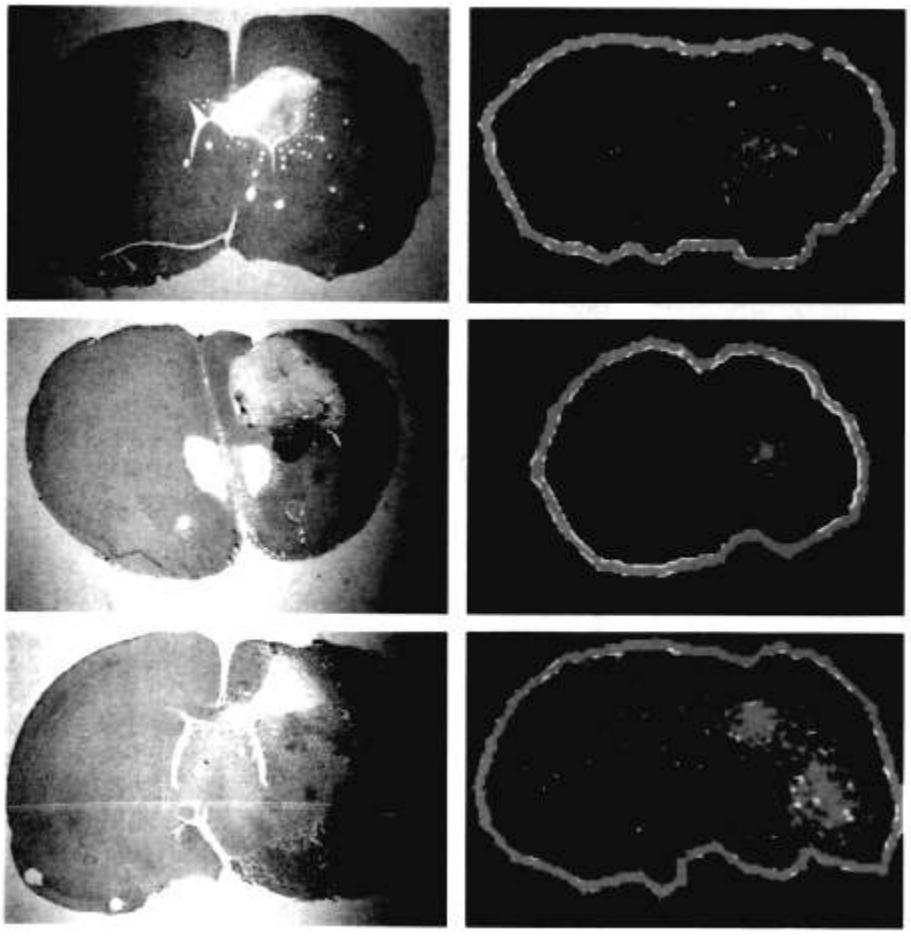

Figure 3. Fischer 344 rats with malignant RG 2 glioma cells inoculated in the caudate nucleus in the brain, and intratumoral ^{201}Tl injections 15, 17 and 19 days after inoculation. The left column shows staining with hematoxylin & eosin of coronal sections through the middle of the tumor area. The right column shows radioactivity measurements with the β-camera in adjacent slices to the ones in the left column from the same animals. The upper row shows total necrosis at the injection site. The middle row shows viable tumor at the edge of the necrosis. The bottom row shows viable tumor at a distance from the original tumor site.

CSF circulation. Further support is given from the β-camera measurements after dissection, where ^{201}Tl activity could be detected in tumor tissue not only at the original tumor site but also in distant tumor growth but not in normal tissue.

In a study by Rao *et al.*[15] it was shown that after intratesticular injections of ^{201}Tl and ^{204}Tl there was a larger radiobiological effect of ^{201}Tl compared with ^{204}Tl for the same average absorbed dose. This enhanced effect was attributed to the fact that ^{201}Tl emits numerous Auger electrons with a very short range giving a very high local energy deposition close to the decay point. In a recent calculation of Goddu *et al.*[16] it has been shown that if ^{201}Tl is localized within the cell nucleus, evenly distributed in the cell or localized on the cell surface, the absorbed dose to the cell nucleus will be 4.2, 1.7 and 0.18 mGy/MBq s, respectively. Thus, an internalization of ^{201}Tl will give a very high absorbed dose to the genetic material in the cell nucleus facilitating a good therapeutic effect. We consider this effect to be the main reason for the selective necroses of the tumor cells in the present study.

Practical questions that arise are how one can assure that ^{201}Tl is injected in the center of the tumor. Even though ^{201}Tl was injected stereotactically there could have been a mismatch between tumor growth and isotope injection. Further, it is not known how far ^{201}Tl can diffuse from the injection site in relation to the injection volume and velocity. ^{201}Tl has a half-life of approximately 3 days. It is not known how many injections and how long intervals between injections are required to achieve an absorbed dose optimal for a necrotizing effect on the malignant glioma cells. However, at the injection sites, the radiation was apparently sufficient to produce a local, circumscribed necrosis. In most animals no tumor tissue was found in the necrotic area. This indicates that irradiation of malignant gliomas is possible with intratumoral injections of ^{201}Tl provided that the deposition and diffusion of ^{201}Tl coincide with the tumor growth.

In some rats tumor growth was found in remote locations separated from the circumscribed necrosis. In three out of four cases there was also some ^{201}Tl in these distant tumors. A possible explanation is that the primary tumor generated metastases, especially in the injection canal which was penetrated repeatedly with the syringe for the ^{201}Tl injections, and that these distant tumors absorbed a lower concentration of ^{201}Tl which was suboptimal for total necrosis.

The present study is planned to continue with more detailed kinetic and dosimetric investigations of ^{201}Tl after intratumoral injections with different doses and time intervals.

Conclusions

(1) Local repeated intratumoral injections of ^{201}Tl in malignant gliomas produced a prolonged retention of radioactivity at the site of injection most likely in the glioma cells, with no uptake in other organs except for the kidneys.
(2) Singular circumscribed necroses were produced with the injection procedure employed, comprising malignant glioma tumor tissue which in most cases was irradiated at the site of injection.
(3) The selective necrotizing effect on the tumor cells is interpreted as mainly due to emission of Auger electrons in the vicinity of the cell nucleus from intracellularly accumulated ^{201}Tl.
(4) The β-camera is a useful instrument for assessment of the distribution of radioactivity at the cellular level in tissue slices.
(5) The results should have implications for the treatment of human malignant gliomas.

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