Project title: **Anatomical and physiological changes of functional capillaries in normal mouse brain tissue after microbeam radiation exposure.**

Introduction

The main limitation of radiotherapy is possible radiation damage to normal tissue adjacent to the tumor. Therefore, in conventional radiotherapy treatment of primary brain tumors e.g., the dose is fractionated over weeks and focused on the tumor. The risk of fractionated radiotherapy is, however, DNA repair and re-growth of tumor cells during treatment. A high single dose would be more efficient, but impossible without serious damage to normal brain tissue. One of the important risks, beside damage to neurons and glial cells, is the breakdown of the blood brain barrier (BBB), which may cause cerebral edema and consequently high intracranial pressure.

A new technique, known as microbeam radiation therapy (MRT) using synchrotron radiation x-rays, has recently been developed and is based on the idea that radiation damage in normal brain tissue can be considerably decreased by spatial microfractionation of the absorbed dose. This would enable treatment of brain tumors with a high single dose: 312 or 625 Gy, which is the peak dose of the microbeams crossfired at the tumor location. Indeed, rats bearing 9L gliomas, show an increased lifespan of several months after MRT without changes in neurological behavior. Several researchers are working on the effect of MRT on normal brain cells. *In the present study, we focused on analyses of the early effects (2 hours – 12 days) of MRT on the microvasculature in the cortex of nude mice by intravital two photon microscopy. The aim of the study was to determine if MRT induces vascular damage, such as BBB breakdown and a decrease of blood volume in normal brain tissue.*

Material and methods

Two-photon microscopy enables analyses of changes of local cerebral blood volume and vascular permeability *in vivo* at capillary level. Up to now, one low and one high radiation doses have been applied: 312 and 1000 Gy. The complete left hemisphere of female nude mice (4 – 6 weeks old) was irradiated at a depth of 3 mm. The size of the 16 microbeams was 25 µm with an interdistance of 200 µm. At different time intervals after MRT (2h, 24h, 48h, 4d, 7d and 12d), changes in vascular volume and permeability in the parietal cortex were measured using respectively, an intravascular fluorescent probe (FITC-dextran, Mw = 70.000) and a diffusible (sulforrhodamine B, Mw = 0.3 kDa). The number of mice was 3 per time interval after MRT. They were anesthetized with a mixture of Xylazin/Ketamin and were placed on the motorized step stage of the microscope after craniotomy (3 mm in diameter). The vascular volume was estimated from z-scans over a maximum distance of 650 µm starting at the top of the brain in the dura mater. The vascular permeability was detected as extravasations of the diffusible probe in the microbeam stripes. After two-photon microscopy or on a different cohort of mice, immunohistochemistry was used to stain all vessels with collagen IV (basal lamina) and functional endothelial cells were detected by PECAM 1. In addition, haematoxylin (nuclei) and eosin (cytoplasm) staining (HE) was applied to detect radiation damage of glial cells and neurons in microbeam stripes.

Results and discussion

From 12 hours until 12 days after MRT, a diffusion of sulforhodamine B in microbeam stripes was observed using a dose of 1000 Gy. No diffusion was detected 1 month after MRT. It seems that the BBB breakdown occurs 12 h and is repaired between 12 and 30 days after irradiation. For all time intervals after MRT, the FITC-dextrans remained in the functional vessels. For a dose of 312 Gy, no diffusion of sulforhodamine B or FITC-dextrans was detected at any time after MRT.

For 312 and 1000 Gy, no important changes in vascular volume were observed for all time intervals after MRT: all microvessels remained functional in the microbeam stripes. The latter was confirmed by the immunohistochemical staining of the basal lamina and the vascular

endothelial cells. A co-localisation of both probes indicates that the vascular endothelial cells are viable and present within the basal lamina. However, this does not exclude a BBB breakdown as observed after a dose of 1000 Gy in the previous paragraph. In the HE stained sections, glial and neuronal cell death was already detectable in the microbeam stripes as soon as 2 h after MRT. Many cells showed pycnosis. Four days after MRT, microbeam stripes appeared empty with a complete disappearance of glial cells and neurons. Finally, no radiation damage was observed in areas in between the microbeam stripes.

Conclusions

With a dose of 312 Gy, no radiation damage to the microvasculature in the microbeam stripes of normal brain tissue was detected. This dose would therefore be a more appropriate for the treatment of gliomas using crossfired microbeams. At this dose, no BBB breakdown was detected for sulforhodamine B (Mw = 0.3 D), however, an increased permeability of the BBB for water is not excluded and will be examined in the next series of experiments.