SINGLE DOSE IRRADIATION OF DEFINED REGION OF RAT BRAIN WITH STEREOTACTIC BRAINLAB SYSTEM

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EGYSZERI NAGY DÓZISÚ PATKÁNY RÉSZAGY-BESUGÁRZÁS BRAINLAB SZTEREOTAKTIKUS RENDSZERREL

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Background and purpose of our study was to develop a precise dose delivery technique for partial brain irradiation of two rats simultaneously.

Methods – Using a self-developed frame stereotactic radiotherapy with single doses of 30-90 Gy was delivered to the frontal lobe of 22 animals. Tolerability and reproducibility of the method were evaluated and dosimetric measurements were conducted to verify the treatment plans. 2, 4 and 6 months after the irradiation magnetic resonance imaging (MRI) scans and histopathological examinations were performed to detect late radiation induced biological changes.

Results – Immobilization device provided excellent reproducibility and tolerability. Dosimetry revealed good correspondence with planned dose distribution, but the measured absorbed dose was 30% lower than the planned dose. During the 6 months follow-up period the procedure related death of subject animals after 30 Gy, 70 Gy and 90 Gy were 0%, 20% and 100% respectively. T2 signal and structural changes on MRI scans found to be dose and time dependent. While 30 Gy caused no detectable structural changes, 70 Gy lead to cystic necrosis in 2 cases after 4 month. Histopathology revealed signs of necrosis on macroscopic examination after 70 Gy in the high dose region involving both frontal lobes, and no obvious microscopic changes in the surrounding area were detectable.

Conclusion – Our technique of rat cranial irradiation using human stereotactic system provided high accuracy of single dose delivery for a pair of small animals, resulting in brain injury in the defined area. This method proved to be a reproducible model for preclinical studies on radiation effects.

Keywords: *radiosurgery; animal model; radiation necrosis; MRI*

Célkitűzés – A vizsgálat célja olyan nagy pontosságú kisállat részagy-besugárzási technika kidolgozása, amellyel egyidejűleg két patkány azonos agyi régiójának szelektív irradiációja végezhető.

Módszer – Saját fejlesztésű immobilizációs rendszert alkalmazva a BrainLab sztereotaktikus besugárzó egységével 30–90 Gy egyszeri dózissal irradiáltuk 22 Wistar-patkány homloklebenyét. A módszer reprodukálhatóságát és tolerálhatóságát értékeltük, valamint a besugárzási tervek ellenőrzése céljából részletes dozimetriai méréseket végeztünk. Az állatokat az irradiáció után 2, 4 és 6 hónappal vizsgáltuk mágneses magrezonancia-képek segítségével (MRI), majd a morfológiai elváltozások értékelését hisztológiai vizsgálattal egészítettük ki.

Eredmények – A két állat egyidejű kezelését szolgáló immobilizációs eszköz kiváló reprodukálhatóságot biztosított és az állatok jól viselték. A dozimetriai mérések jó egyezést mutattak a tervezett dóziseloszlással, azonban a mért abszorbeált dózis 30%-kal alacsonyabb volt a tervezettnél. A megfelelő dózis-korrekció után 30, 70 és 90 Gy dózisszinteken 0, 20 és 100%-os mortalitást detektáltunk. A T2-szignál és a strukturális MRI-változások dózis- és időfüggőnek bizonyultak. Még a 30 Gy nem okozott strukturális változásokat, a 70 Gy dózissal besugarazott csoportban azonban két esetben is cisztikus nekrózis alakult ki, négy hónappal a besugárzás után. A szövettan ≥70 Gy dózisnál, mindkét frontális lebeny nekrózisát mutatta, miközben a környező területeken nem volt értékelhető mikroszkópos elváltozás.

Következtetés – Az általunk kialakított patkány részagy-besugárzási technika humán sztereotaktikus rendszerben, nagy pontosságú, egyszeri dózisleadást biztosít egyidejűleg két kisállat számára, kifejezett agyi károsodást okozva az előre meghatározott térfogatban. Ez a módszer jól alkalmazható a sugárhatás kísérletes tanulmányozására.

Kulcsszavak: *sugársebészet, kisállatmodell, sugárnekrózis, MRI*

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Radiosurgery (RS) has been widely used treatment method of malignant tumours of central nervous system (CNS). It allows the delivery of highly conformal single dose to small focal regions without affecting surrounding brain structures. However, neuropathological changes and late clinical side effects such as demyelination and necrosis may occur, which constrains the use of RS[1, 2]. Although these late reactions of normal human brain tissue have been intensively investigated, its pathomechanism is not well understood yet. In spite of many inherent limitations, animal models are essential tools in evaluation and understanding of radiation induced brain injury. It is well known from previous studies that in murine models – due to lower radiosensitivity – larger doses are needed to induce necrosis of brain tissue than doses used in human medicine[3]. Delivering such high doses to selected brain structures requires stereotactic techniques and in case of large number of animals it is a time consuming process. The vast majority of available data regarding stereotactic irradiation of small animals are from studies based on gamma knife and only few papers are dealing with Linear Accelerator (LINAC) based conformal RS. The main aim of our study was to develop a conformal method of murine cranial radiotherapy which enables the reproducible, precise delivery of high doses to multiple subject animals simultaneously. This model could serve as a useful tool for conducting preclinical studies to assess radiotherapeutic response of brain tissue with or without additional treatment.

Materials and methods

ANIMALS

In our study twenty-two female Wistar rats weighting 350-400 g were irradiated with various doses. The experiment was performed with the approval of the Ethical Committee of the University of Pécs in accordance with European Communities Council Directive of 24 November 1986(86/609/EEC). Rats were kept two per cage at temperature 22±1 C°, in 12 hours light/dark cycles and they had free access to standard pellet rodent diet.

ANAESTHESIA

Prior to irradiation and computed tomography (CT) scanning, animal subjects were intraperitoneally anaesthetised with 350 mg/kg chloral hydrate. During the magnetic resonance imaging (MRI) examinations, rats were intubated and anaesthetised with 1.5% isoflurane (Forane®; Abbott Laboratories Hungary Ltd., Budapest, Hungary) in 70/30 mixture of NO_2 and O_2.

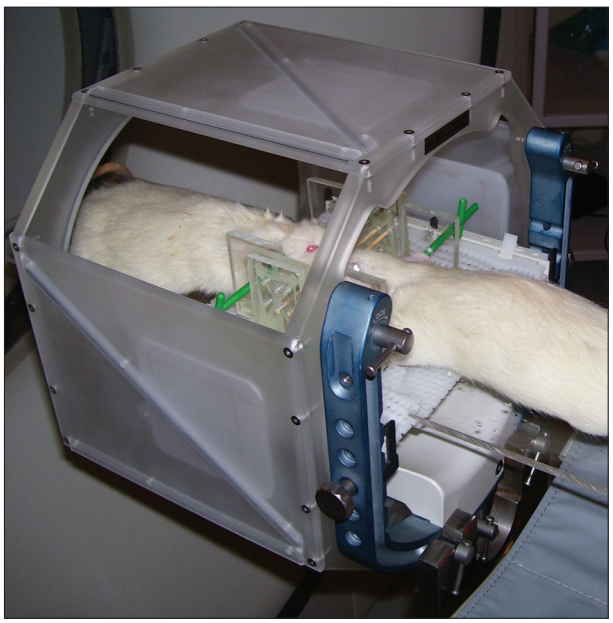

Figure 1. *The self-developed plastic frame, that enabled the irradiation of two animals simultaneously, was fixed to the stereotactic frame of BrainLab system*

IMMOBILIZATION

During CT scans performed for treatment planning purposes and during irradiation a pair of subject animals were fixed simultaneously in prone position, on a plastic frame with bite bars and both-sided earplug bars. Rats were placed in front of each other with 5 mm lateral shift, so thus the heads of two animals including the desired treatment volumes were within 10 mm distance. The baseboard of self-developed plastic frame was fixed with thermoplastic materials to the stereotactic frame (BrainLab radiosurgery, BrainLab, Munnich, Germany), which was attached to the CT table and the LINAC table (Precise Treatment System™, Elekta, Stockholm, Sweeden) as in case of human stereotactic radiotherapy (**Figure 1.**).

TREATMENT PLANNING

As the first step of 3 dimensional (3D) conformal radiotherapy native CT slices 1 mm of width were performed (Siemens CT Scanner, Siemens Medical Solution, Erlangen, Germany). Before the scanning a pair of animals was positioned in the head-immobilizer, fixed to the BrainLab frame.

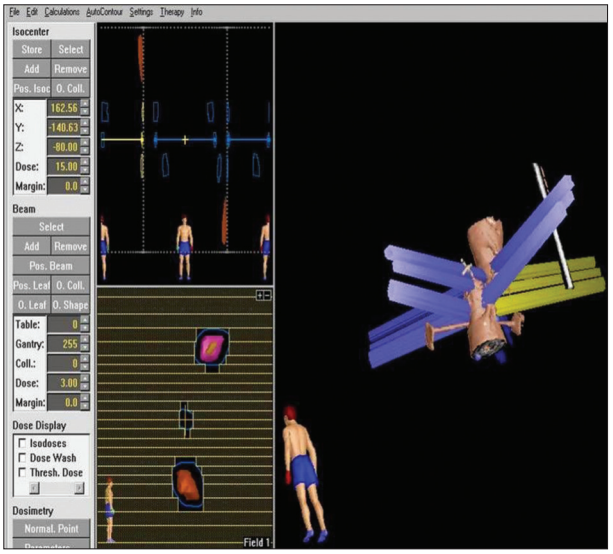

Figure 2. *Radiotherapy plan with single isocenter comprised of 5 equally weighted, coplanar beams at the 270°, 315°, 0°, 45° and 90° gantry position*

Stereotactic box of BrainLab system, containing metal markers for orientation in space and positioning of isocenter, was attached to the frame during the CT examination. The same rats were MRI scanned with 3.0-T clinical MRI scanner (Magnetom TIM Trio, Siemens Medical Solution, Erlangen, Germany). The resultant DICOM images were then imported to BrainScan 5.31 planning system and CT and MRI scans were fused. Left frontal lobes of both animals were contoured with help of rat brain MRI template[4]. To irradiate both target volumes at same time conformal radiotherapy plan with single isocenter situated between the heads of two rats was constructed. The treatment plan comprised of five equally weighted, coplanar beams at the 270°, 315°, 0°, 45° and 90°gantry position **(Figure 2.)**. Dose distribution was calculated with pencil beam algorithm, and the planned dose was prescribed to the 80% isodose level. Dose-histograms and isodose curves were generated and evaluated for this plan and 3D coordinates of the isocenter were outputted. Besides, printouts containing the position of the isocenter and the projections of treatment fields on all sides of the stereotactic box were made.

IRRADIATION

Before irradiation printouts holding various aspects of treatment fields and defining the position of the isocenter were fixed to the BrainLab box. Using these signs on stereotactic box isocenter set up was performed with a laser system following anaesthetization and positioning of a pair of animals. Irradiation was delivered with 6 MV photons (Electa Precise TS LINAC) and micro-multileaf collimator (BrainLab) which characterized with leaf-width of 3 mm. Before the treatment light fields were checked and electronic portal images were taken to ensure the accuracy of the isocenter set up. At one occasion all fields corresponding to a pair of rats were irradiated according the previously prepared plans with total doses of 30 Gy (n=6 animals), 70 Gy (n=10 animals) and 90 Gy (n=6 animals) respectively.

DOSIMETRY

For dosimetry the rat head was simulated by a phantom made of water equivalent bolus material. This phantom was CT scanned and using its data the same plan was made as in the animal model. To verify the treatment plan measurements were performed with X-ray film (Kodak EDR2) placed in the plane of the radiation fields and with dosimetry diode (Scanditronix PFD) inserted into the planned target volume the phantom head **(Figure 3.)**.

MAGNETIC RESONANCE IMAGING

Under anaesthesia, three animals of each dose group underwent follow-up MRI 2, 4 and 6 months after the irradiation. MRI studies were performed with 3.0-T clinical MRI scanner (Magnetom TIM

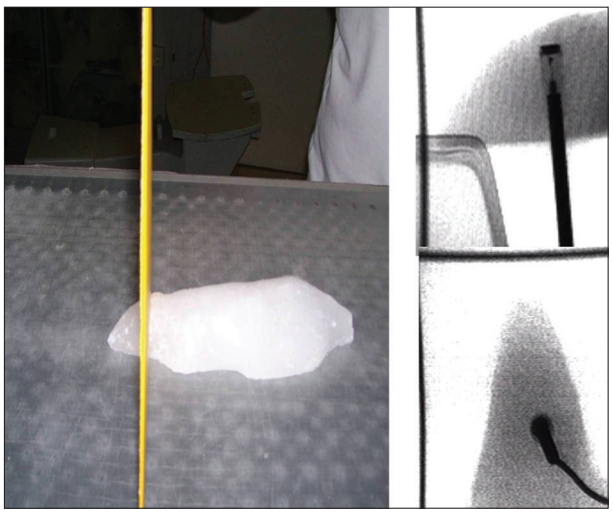

Figure 3. *Phantom made of water equivalent bolus material was used to perform dosimetry with X-ray film (Kodak EDR2) and dosimetry diode (Scanditronix PFD) inserted into the planned target volume*

Trio, Siemens Medical Solution, Erlangen, Germany) with a filed gradient strength of 40 mT/m equipped with a commercial radiofrequency (RF) coil with an inner diameter of 40 mm (Siemens Medical Solution, Erlangen, Germany). The head of each rat were fixed in a custom-built plastic holder and placed through a loop RF coil. The scanning procedure contained T1-, T2-weighted and diffusion measurements.

HISTOPATHOLOGY

At the end of the MRI experiments, the animals were deeply anaesthetised with diethyl-ether and perfused transcardially with 250 ml of 0.1 M phosphate-buffered saline (PBS) pH 7.4; followed by 300 ml of fixative. The brains were rapidly removed, postfixed in 4% paraformaldehyde for 1 hour, and then blocks were routinely embedded in paraffine, and serial sections were cut on a sliding microtome in the coronal plane at a thickness of 10 mikrom. These sections were processed for hematoxylin-eosin staining for the general overview of the gross changes and Luxol Fast Blue – Direct Red 80 staining for the better visualisation of the myelinated axons.

Results

TREATMENT THROUGHPUT

The conformal treatment plan generated by the stereotactic planning system provided homogeneous coverage of the two targets with average 134 mm^3 (range 118-162 mm^3) volume, which were encompassed by the 80% isodose, meanwhile 39% of the normal rat brain being outside the target volume received 20% of the prescribed dose. Immobilization device provided good reproducibility of subject animal positioning. Visual inspection of light fields and electronic portal images of treatment fields did not revealed significant alterations requiring the modification of initial setups. Pretreatment procedures, which include the positioning of a pair of animals, couch and collimator setup took 4-5 minutes, while the delivery of 30 Gy, 70 Gy and 90 Gy required 29, 51 and 65 minutes respectively. With intraperitoneally administered chloral hydrate 5-15 minutes needed to achieve full anaesthesia, which was maintained for 70-90 minutes covering the whole irradiation time in all cases. None of the irradiation procedures was interrupted because of insufficient anaesthesia. Irradiation regimens were tolerated well by animal subjects and no complications were observed related to immobilization device.

DOSIMETRY

Digital evaluation of darkening of the X-ray film exposed to radiation fields has revealed a good correspondence with the planned dose distribution. While the dose calculated by the treatment planning system required correction (Dx0.7) on the basis of measurement by dosimetry diode.

SUBJECT OUTCOME

One mortality attributed to intraperitoneal anaesthesia occurred. During the follow-up period survival rates depended on the dose which had been delivered to the frontal lobe. All of the subject animals that received dose of 90 Gy died between three and 35 days after the irradiation (procedure related mortality rate: 100%, median survival: 13.7 days). One animal died 4 days and another 43 days following irradiation with dose of 70 Gy (procedure related mortality rate: 20%). None of the animals received dose of 30 Gy died during the entire 6-month-long follow-up period (procedure related mortality rate: 0%). We experienced no weight loss or any change in eating habits of the irradiated rats. All animals, except those had died shortly after radiation procedure, developed hair loss from two to three months following irradiation independent from the radiation dose, while in case of three animals irradiated with 70 Gy cataracts appeared after six months.

MRI FINDINGS

T2 signal values and structural changes in the frontal lobes were evaluated by MRI at 2, 4 and 6 months following irradiation. MRI finding were dose- and time-dependent. The rats irradiated with dose of 30 Gy had no structural changes in frontal lobe during the first 6 months after irradiation. The response to dose of 70 Gy and the onset of necrosis was not uniform. In case of two rats we examined repeatedly with MRI clear cystic necrosis was visible in the frontal lobe along the high dose region after 4 months, which did not change afterwards (**Figure 4.**). In the brain of the other animal no structural change was documented during the first 6 months. None of the rats irradiated with planned dose of 90 Gy underwent MRI examination, because these animals had died before the first follow-up scanning was planned. No oedema, mass effect could be detected by MRI.

HISTOPATHOLOGICAL FINDINGS: MACROSCOPIC CHANGES

On the macroscopic examination of the brain of the rat irradiated with 70 Gy, an obvious necrosis (emollition) was observable, from the frontal pole until the optic chiasm, involving the olfactory bulbs, bilaterally **(Figure 5.)**. In the light microscopic examination of the slides from the brain of the rat irradiated with 70 Gy, no obvious change can be detected in the amount of fibres, thickness and general structure of the callosal body compared with that of the control animal (without any irradiation) **(Figure 6.)**.

Discussion

We have developed and evaluated a conformal method of partial rat CNS irradiation. To precise delivery of high doses to small target volumes in the majority of previous studies gamma-knife was used[5–8]. LINAC based studies mainly have utilised non-targeted dose delivery resulting from full body radiation fields with selective shielding of extracranial parts[9, 10] or one small standard field with bolus above the skull[11]. More precise and more conformal dose delivery could be achieved with arc therapy applying cylindrical collimators which enables irradiation of sub-regions of one subject animal skull[12–14]. Our method is the first approach that enables the conformal irradiation of selected brain regions in multiple animal targets at the same time. Although the simultaneous irradiation of more than one rats had been described by *Vinchon-Petit* et al.[10], it was not a conformal method, since the whole brain of four animals were treated with uniform radiation fields of 15×15 cm at 100 cm source axis distance[10]. We could achieve conformal, simultaneous irradiation with the use of special immobilization device and BrainLab stereotactic system including micro-multileaf collimator.

In previous studies usually plastic frames with bite bars and both-sided earplugs were used for immobilization purposes[8, 11]. Our self-developed frame consists of the same structural parts which provide a good fixation of subject animals. Furthermore, it enables the immobilization of two rats so that their brain is within 10 mm distance, thus both brains can be covered with the aperture of BrainLab's micro-multileaf collimator. Micro-multileaf collimator can not only improves conformal character compared to cylindrical collimators, but also enables to form two treatment fields at the same time in all gantry position.

Precise delineation of frontal lobe was allowed

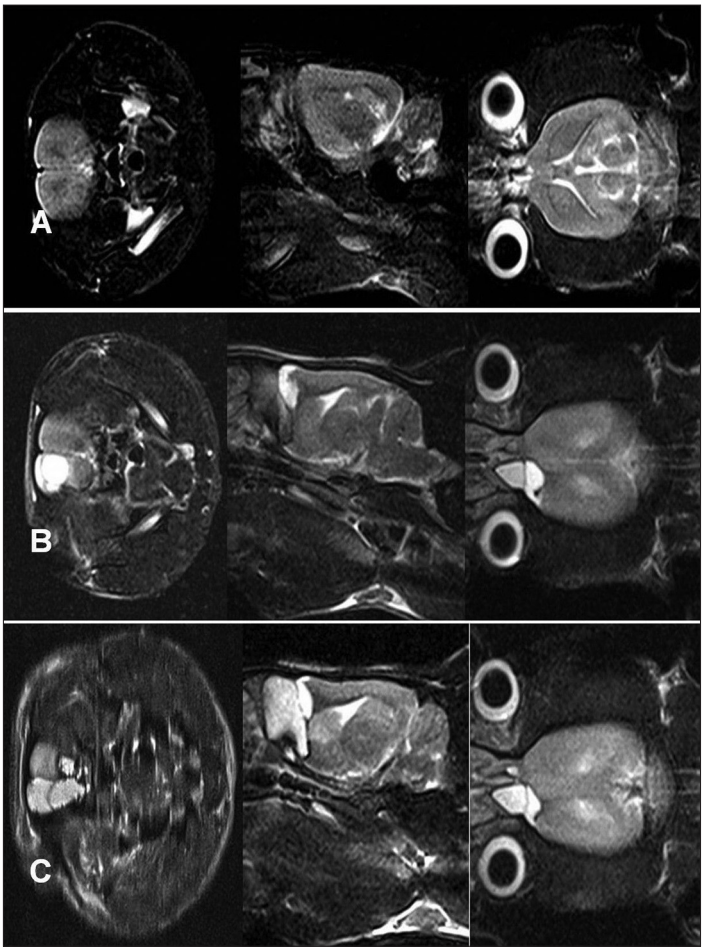

Figure 4. *Following irradiation with dose of 70 Gy T2 weighted MRI images revealed no changes at 2 month* **(A)***, while cystic necrosis of frontal lobes were detected at 4 month* **(B)** *and 6 month* **(C)**

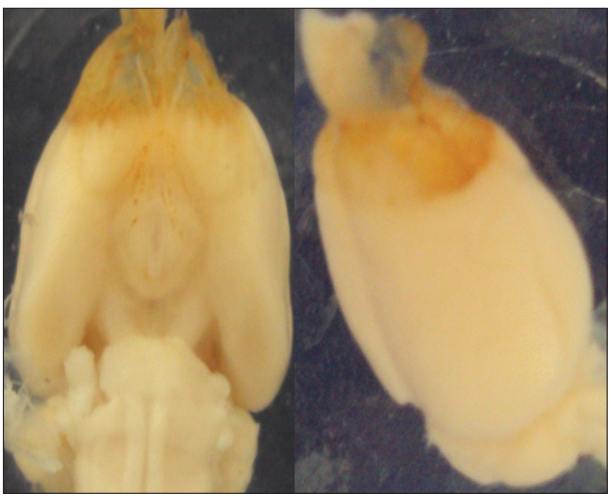

Figure 5. *On the macroscopic examination of the brain of the rat irradiated with 70 Gy, an obvious necrosis (emollition) corresponding to target volume was observable*

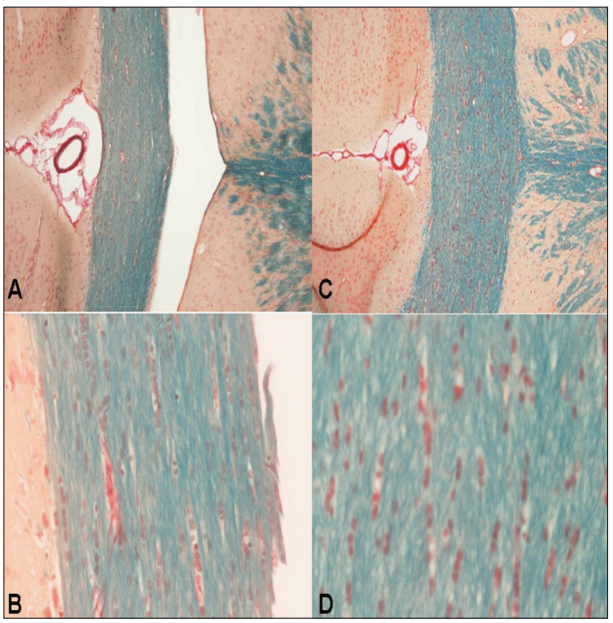

Figure 6. *Light microscopic images of irradiated (with 70 Gy* **A** *and* **B***) and control (***C** *and* **D***) animals, genu of the callosal body, coronal sections, in the level of the optic chiasm. Myelin staining (Luxol Fast Blue), magnification 40× (***A** *and* **B***) and 160× (***C** *and* **D***)*

by BrainScan System that provides fusion protocol of the MRI of rat brain and pre-treatment CT slices. Similar fusion protocol was used by *Stecken* et al.[11], while in other studies the target area definition based on stereotactic coordinates taken from rat brain atlas[7-8, 14].

In a former study delivering 20-50 Gy with 15 MV LINAC took 20-120 minutes[13], while in our study the overall treatment time for the irradiation of two animals with doses of 30 Gy, 70 Gy and 90 Gy were 29, 51 and 65 minutes respectively. Although our method requires significant amount of labour, it is more time-efficient than pervious LINAC based techniques.

We constructed conformal, multi-beam plans and tried to determine 3D dose distribution, as well as dose-volume data, while majority of previous studies used only rough dose calculations. However, our dosimetry results suggest that dose-volume data provided by BrainLab planning system should be dealt with caution in case of small fields and small animal targets. While film dosimetry implies that there is good match between measured and calculated dose distribution in the plane of the radiation fields, diode dosimetry in rat phantom revealed 30% lower doses than planned. This significant difference can be resulted from inadequacy of both the planning system and the dosimetry equipments used in human medicine for predict doses in small targets. Nevertheless, for evaluation of biological effects of radiation in our murine model it is seemed to be reasonable to use a correction factor of 0.7.

Radiation regimens were well tolerated with minimal acute side effects and peri-procedural mortality. The vast majority of treatment related mortality was experienced in the group of animals that had been irradiated with dose of 90 Gy, whereas the animals received 70 Gy and 30 Gy were able to survive the 6 months follow-up period. This is in line with the result of *Liscak* et al.[7] who found that irradiating both hippocampus, with 100 Gy or higher doses lead to the death of subject animals within 4-5 months[15]. In contrast, others found that after 100 Gy delivered with 15 MV LINAC to 3.7 mm or 4.7 mm spherical target volume in the right frontal lobe of rats only two out of 12 animals died during the 19 months observation period[14]. These data suggest that treatment related mortality rate depends not only on radiation dose, but also on location and size of target volume. Radiation induced morphological changes demonstrated with repeated control MRI scans have proved to be dose-, time- and location-dependent as well. In our model visible cystic necrosis of frontal lobe appeared from 4 to 6 months following irradiation with 70 Gy, whereas with dose of 30 Gy we could not detect any structural changes during the follow-up period. Similarly no change was found during the first three months by other authors exposing the whole hippocampus to 25 Gy, 50 Gy, 75 Gy. After six months 50 Gy did not induce MRI changes consistently, while necrosis were demonstrated after 75 Gy irradiation so the lesion developed within three and six months[6-7]. Single doses of 150 Gy and 100 Gy can produce necrotic lesions in the hippocampus within one to three months[3, 16], but in the present study such high doses to frontal lobe were not tolerable. In line with our results in former studies within six months follow-up period necrotic changes started appearing at doses higher than 60 Gy[7, 17], although after 20 months of follow-up lower doses, such as 25-50 Gy delivered to the right frontal lobe of rats were demonstrated to cause MRI changes in T1 and T2 relaxation time[12, 18]. In our study macroscopic examination of removed rat brain confirmed the time- and dose-dependent necrotic changes described by MRI. In the frontal lobe of the animals received 70 Gy necrosis developed after 4 months of follow-up, while lower doses did not cause any detectable changes. Based on previous studies histopathological structural changes – loss of cell number, demyelination – can be expected in dose range of 50-100 Gy[6, 9, 11, 16], however on the light microscop-

ic examination we could not identify any alterations around the necrotic areas.

In conclusion, we have developed a well tolerable, conformal, LINAC based method of murine cranial irradiation which enables delivery of high doses to a defined bilateral part of the brain for a pair of animals simultaneously. In our investigation the human stereotactic system could be applied for the irradiation of small animal targets, but correction of the uncertainty of the planning system developed for clinical use and careful dosimetry has to be performed. Our method is found to be geometrically precise and it can induce biological changes in the targeted brain area providing a reproducible model for preclinical animal studies on radiation effects and its modifiers.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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