Sonodynamic Therapy Consisting of Focused Ultrasound and a Photosensitizer Causes a Selective Antitumor Effect in a Rat Intracranial Glioma Model

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Abstract. In this study we sought to determine the optimal focused ultrasound acoustic conditions with photosensitizers for the ablation of experimental intracranial glioma in rats. Materials and Methods: Normal rat brains were sonicated via a transducer placed on the dural surface with or without a prior intravenous injection of the photosensitizer Rose Bengal (50 mg/kg of body weight). The ultrasound intensity was varied to 25, 110 or 150 W/cm², and the duration of irradiation was 10 s, or 1, 3, or 5 min. In experimental intracranial gliomas, one week after inoculation of C6 rat glioma cells in the rat brain, the rat brain was sonicated through a 10 mm-diameter craniotomy. Results: A selective antitumor effect against cerebral glioma while sparing normal brain tissues was achieved by sonodynamic focused therapy consisting of focused ultrasound at 25 W/cm² at 1 MHz for 5 min and Rose Bengal (50 mg/kg of body weight). The areas of tumors in sham-operated rats and in rats that received sonodynamic therapy without and with Rose Bengal at an intensity of 25 W/cm² for 5 min were 19.53±3.89, 10.64±2.21 and 3.01±1.74 mm², respectively. The tumor area was significantly smaller in the ultrasound therapy groups than in control non-treated animals (p=0.002). There was no significant temperature change in tumor tissues during sonication with 25 W/cm² at 1 MHz. Conclusion: This is the first report to demonstrate the usefulness of sonodynamic therapy consisting of focused ultrasound and photosensitizer for the treatment of experimental malignant glioma. Photodynamic therapy (PDT) with laser light of an appropriate wavelength has been extensively investigated in laboratory studies for the treatment of a variety of brain tumors, particularly gliomas, and has been applied in clinical trials (1). The efficacy of this therapy is based on the uptake of photosensitizer by neoplastic tissue, its clearance from surrounding brain tissue and the timing and placement of photoactivating sources (2, 3). The main advantage of PDT lies in its ability in combination with photosensitizer to select tumor cells that are infiltrating brain parenchyma (4, 5). Unfortunately, it is quite difficult in PDT to select the optimal individualized treatment conditions of light-dose volume and geometry, and to treat massive or deep-seated tumors due to the poor penetration of optical beams into tissues (6). This has severely compromised the curative ability of PDT. Ultrasound can penetrate intervening tissues and deliver its energy to non-superficial areas, while maintaining the ability to focus its energy into a small volume (7). Focused ultrasound delivers ultrasonic energy with resultant heat and cavitation to achieve discrete tissue destruction without damaging intervening tissue or cells in the immediate vicinity of the focal area (8, 9). This is a unique advantage over electromagnetic modalities, such as laser and microwave, for the noninvasive treatment of deep-seated tumors. Focused ultrasound therapy has been extensively investigated in laboratory studies in the treatment of liver (10, 11), prostate (12) and bladder tumors (13), and has been used in clinical trials to treat these tumors (14-16). Focused ultrasound has also been used for the selective destruction of brain tissue in neurosurgical research since the 1940s (17-19). Umemura et al. reported that hematoporphyrins, which are photosensitizers, can enhance the antitumor effect of ultrasound and proposed sonodynamic ultrasound therapy as a new modality for treating tumors (20, 21). The input power can be reduced to a level at which potentially hazardous effects to surrounding tissues are avoided (22). Using this approach, special drugs, given to the patient in advance, are activated by focused ultrasound in the tumor. Several materials besides porphyrins, such as certain xanthene dyes (20) and antitumor...
drug (23, 24), have been found to be activated by ultrasound and show a significant antitumor effect. Furthermore, a series of investigations appear to show that the responses of malignant cells to ultrasound are not identical to those of normal cells, in that cancer cells are more prone to being killed by ultrasound (25). Human malignant glial tumors comprise the majority of primary intracranial tumors, which are often characterized by rapid growth and invasiveness into surrounding normal brain tissue (26). This diffusely infiltrative nature of malignant gliomas is one of the major obstacles to successful local resection, which in turn causes the failure of curative treatment, with most recurrences occurring at the site of the original tumor (27). This difficult clinical situation has stimulated interest in additional approaches to the treatment of malignant glioma. A more aggressive local therapy is required to eradicate unresectable (nests) of tumor cells invading adjacent normal brain tissue. Focused ultrasound with photosensitizers that enhance its antitumor effect seems to be attractive for the selective killing of tumor cells in malignant glioma. In this study, we sought to determine the optimal focused ultrasound acoustic energy and duration for the ablation of experimental brain tumor without damaging normal brain tissue in rats.

Materials and Methods

Preparation of a sonodynamically active agent (Rose Bengal). Rose Bengal (disodium tetraiodo tetrachloro fluorescein), a type of xanthene dye, was purchased from Wako Chemical Company (Tokyo, Japan). The material was supplied as a sterile solution at a concentration of 5 mg/ml in saline. The dye was filtered using a 0.22 µm pore filter and stored in the dark until use.

Ultrasound source. The air-back ultrasound transducer used a lead-zirconate-titanate piezoelectric ceramic disk of 8 mm in diameter and was tightly bonded to an aluminum layer with an epoxy adhesive that expanded very little with heat (Hitachi Ltd, Tokyo, Japan) (28). The transducer was cooled by circulating water to keep the temperature at 25˚C during sonication. The overall resonant frequency of the transducer was 1.04 MHz. Sine waves were generated by a wave generator (model MG442A; Anritsu Electric, Kanagawa, Japan) and amplified by an RF amplifier (model 210L; ENI, USA) (28). The sinusoidal drive signal of the transducer was monitored by an oscilloscope during ultrasound exposure. The output acoustic pressure was measured in degassed water 7 mm from the transducer surface using a 1-mm-diameter polyvinylidene difluoride needle-type hydrophone (Medicoteknisk Institute, Denmark). The spatial average intensity was calculated by scanning the probe, from 2-3 mm axially and 1-2 mm laterally, to eliminate the effect of ripples in the field due to Fresnel diffraction. The target depth of the tight focus was approximately 4 mm from the surface without attenuation in overlying tissues. The measured intensity was approximately proportional to the square of the peak-to-peak driving signal voltage used for the exposure. In this experiment, the transducer was driven at a voltage corresponding to a specific free-field intensity, which was considered the ‘peak focal intensity’ of ultrasonic exposure.

Sonodynamic effect in normal rat brain tissue. Adult male Wistar rats (180 to 250 g; Kyudo, Ltd., Kumamoto, Japan) were used for this experiment. The rats were anesthetized intraperitoneally with sodium pentobarbital (40 mg/kg) and placed in a stereotactic frame. The scalps of the rats were shaved and swabbed with alcohol, and a 2-cm midline incision was made. The pericranium was removed from the cranial surface. To accurately position the ultrasound transducer on the brain surface, a 10-mm-diameter craniotomy was performed over the left hemisphere using a high-speed dental drill. Care was taken to not damage the dura mater or the brain surface. The transducer was placed firmly on the dural surface, which was previously coated with gelatin. The brain was sonicated in a standing-wave mode at the peak focal intensity 10 min after the intravenous administration of Rose Bengal (10 mg or 50 mg/kg body weight). The target lesion for focused ultrasound was the left caudoputamen. In control groups, the same dose of saline was injected intravenously before sonication. Rose Bengal doses were determined according to the method of Watson et al. (29). The peak focus intensity was 25, 110 or 150 W/cm² at 1 MHz, and the exposure duration was 10 s, or 1, 3, or 5 min. Five to seven rats were used in each experiment. During sonication, the transducer was cooled by circulating water at 25˚C. After treatment, the wound was closed with 3-0 nylon. The animals were killed 24 h later by an overdose of sodium pentobarbital. The brains were removed, fixed in 10% formaldehyde, sectioned through the area of irradiation, and stained with hematoxylin and eosin. For each rat, the slide that showed the greatest extent of cerebral damage was used for microscopic measurement of the length (L) and width (W) of the lesion. The size of the lesions was measured using image analysis software (NIH Image for Macintosh, USA). To examine the long-term chronic effect of sonodynamic therapy at 25 W/cm² at 1 MHz with an exposure duration of 5 min, the experiment was repeated and the animals were killed one and two months later by an overdose of sodium pentobarbital. The brains were removed, fixed in 10% formaldehyde, sectioned through the area of irradiation, and stained with hematoxylin and eosin. Five rats were used in each experimental group.

Brain temperature measurement. In another 20 rats, the brain temperatures were monitored by stereotactically inserting a thermocouple tip (PTE-300, Unique Medical, Tokyo, Japan) into the center of the lesion before and during sonication. Ultrathin 150 µm-diameter bare fiber thermocouples were used for in vivo thermometry. The rectal temperature was also monitored. Surgery was performed as described above. Ten minutes after the intravenous administration of Rose Bengal (50 mg/kg body weight) (n=5), or the same dose of normal saline (n=5), the brain was sonicated at a peak focal intensity of 25 and 110 W/cm² at 1 MHz for 5 min. The animals were killed 24 h later by an overdose of sodium pentobarbital. The brains were removed, fixed in 10% formaldehyde, sectioned through the area of irradiation, and stained with hematoxylin and eosin to confirm the ultrasound-produced lesion and proper insertion of the temperature probe.

Treatment of experimental C6 rat brain tumor. C6 rat glioma cells were obtained from the Human Science Research Resources Bank (Osaka, Japan) and maintained in Ham’s F-10 medium supplemented with 2.5% fetal calf serum (Calf serum; Hyclone Lab., USA) and 15% horse serum (Horse serum donor herd; Invitrogen Japan, Tokyo, Japan), penicillin G (100 IU/ml) and streptomycin (100 mg/ml) in an atmosphere of 95% humidified air and 5% CO₂ at 37°C. To produce experimental rat brain tumors in the brains of adult male
Wistar rats, a cell suspension of C6 cells in PBS was injected into the brain. Briefly, animals were anesthetized intraperitoneally with sodium pentobarbital (40 mg/kg of weight) and immobilized in a stereotactic apparatus. The cells (5x10^6 cells/5 μl in 1.5% methylcellulose/PBS) were inoculated into the caudoputamen in the left hemisphere (1 mm posterior and 4 mm lateral to the bregma; 5 mm deep) of the rat brain over a period of 5 min with a Hamilton syringe. One week after C6 inoculation in the rat brain, the rat brain was sonicated through a 10-mm-diameter craniotomy in the left hemisphere. The peak focus intensity was 25 W/cm² at 1 MHz, and the exposure duration was 5 min, with or without Rose Bengal. (50 mg/kg body weight) intravenously administered 10 min before sonication. Five rats underwent a sham operation which consisted of an inoculation of C6 glioma cells, craniotomy for sonication and placement of the transducer on the dural surface for 10 min without sonication. The animals were killed 7 days later by an overdose of sodium pentobarbital. The brains were removed, fixed in 10% formaldehyde, paraffin-embedded, and sectioned through the area of irradiation. The sections (4 μm) were stained with hematoxylin and eosin, and the tumor was examined microscopically. For each rat, the slide that showed the greatest extent of tumor was used for microscopical measurement of the length (L) and width (W) of the lesion. The largest tumor areas were measured using image analysis software (NIH Image for Macintosh, USA). Intratumoral temperatures were monitored by stereotactically inserting a thermocouple tip (PTE-300, Unique Medical, Japan) into the center of the sonicated region before and during sonication. All protocols for animal experiments were approved by the Animal Subjects Committee Institutional Review Board of Fukuoka University School of Medicine.

**Statistics.** A two-way analysis of variance (ANOVA) was used to compare the produced lesion rate and the change in brain temperature between the groups with and without Rose Bengal. In addition, Student’s *t*-test was used to compare the sizes of lesions and tumors between the two groups. All of the data were analyzed using a contemporary statistical package (SPSS 12.0J; SPSS Inc, Chicago, IL). *P*<0.05 was taken as the level of significance for all tests.

**Results**

**Impact of sonodynamic effect in normal rat brain tissue.** The left caudoputamen was sonicated at the peak focal intensity with and without intravenous administration of Rose Bengal (10 mg and 50 mg/kg body weight). When the peak focus intensity was 25 W/cm² at 1 MHz, there was no mechanical or heat damage in rat brain regardless of the duration of exposure for up to 5 min. Histological examination revealed no lesion with 25 W/cm² at 1 MHz for 5 min of exposure (Figure 1A), 25 W/cm² at 1 MHz for 5 min of exposure with 10 mg/kg Rose Bengal (Figure 1C), or with 25 W/cm² at 1 MHz for 5 min of exposure with 50 mg/kg Rose Bengal (Figure 1E).

When the peak focus intensity was increased to 110 W/cm² at 1 MHz with 3 min of exposure, a zone of sharply delineated coagulation necrosis was observed in the caudate putamen in 2 out of 6 rats with ultrasound exposure alone, in 5 out of 5 rats with ultrasound exposure and 10 mg/kg Rose Bengal, and in 5 out of 5 rats with ultrasound exposure and 50 mg/kg Rose Bengal. When the duration of exposure was extended to 5 min, a lesion was observed in 5 out of 6 rats with ultrasound exposure alone (Figure 1B), in 6 out of 7 rats with ultrasound exposure and 10 mg/kg Rose Bengal (Figure 1D), and in 6 out of 6 rats with ultrasound exposure and 50 mg/kg Rose Bengal (Figure 1F). The central part of the lesion contained only a few remnants of cells and a small amount of hemorrhage; hemorrhage was markedly greater in animals that had received 10 mg/kg Rose Bengal (Figure 2A). The change became remarkable when it made the applied dose of 50 mg/kg Rose Bengal. Swollen and fragmented myelin and pyknotic glial cells were observed within the margin. Intervening cerebral cortex in the immediate vicinity of the focal zone did not show any significant damage (Figure 2B). These findings were consistent in each group. The incidence of lesions increased with the intensity and duration of exposure. The areas of lesions in animals with and without Rose Bengal at an intensity of 110 W/cm² for 5 min were 1.84±0.18 and 4.47±0.70 mm², respectively, and this difference was statistically significant (*p*=0.01). When the peak focus intensity was increased to 150 W/cm² at 1 MHz with more than 1 min of exposure, a lesion was seen in the caudate putamen in all of the rats regardless of the use of Rose Bengal (data not shown).

**Long-term effect of sonodynamic therapy in normal rat brain tissue.** To determine the proper peak focus intensity and exposure time for the treatment of experimental rat intracranial glioma, we examined the long-term chronic effect of sonodynamic therapy at 25 W/cm² at 1 MHz with an exposure duration of 5 min. Under these conditions, there were no ultrasound lesions in the acute stage, 24 h after sonication. There was also no coagulation necrosis or demyelination in the target area of rat brain one and two months after sonication (Figure 3). Thus, these conditions for ultrasound treatment were considered to be safe for normal rat brain and were selected for tumor treatment.

**Temperature change during sonication.** It has been reported that all invasive temperature sensors measure higher tissue temperatures during high intensity focused ultrasound than the actual tissue temperature (15, 30). The ultrasound absorption of bare fiber thermocouples was tested before in in vivo application as described elsewhere (28). The thin (sensor diameter, 150 mm) thermocouple yielded accurate results. It has also been reported that thin bare fiber thermocouples yield the lowest measuring error (15, 30). We used thin 150 μm-diameter bare fiber thermocouples for in vivo thermometry in normal rat brains and experimental intracranial gliomas in rat brains. Figure 4 shows the heating curves at the center of the brain lesion before and during ultrasound irradiation. Significant temperature change was not detected during sonication by 25 W/cm² at 1 MHz with
or without Rose Bengal pretreatment (50 mg/kg of body weight). When the peak focus intensity was increased to 110 W/cm² at 1 MHz, the increase in brain temperature in the center of the lesion during sonication reached a plateau within 2 min and was close to 50°C with 110 W/cm² at 1 MHz. There was no significant difference in the temperature change between the groups with and without 50 mg/kg Rose Bengal. The rectal temperature did not change from that before sonication and remained close to 36°C. These results suggest that the mechanism of the effect of sonodynamic therapy consisting of focused ultrasound and a photosensitizing agent (Rose Bengal) includes not only a thermal effect but also a non-thermal cavitation effect in the normal rat brain (data not shown).
Figure 3. Long-term effects of sonodynamic therapy in rat normal brain tissue. Hematoxylin and eosin (A, C), and Luxol fast blue (B, D) staining of paraffin-embedded sections. No coagulation necrosis or demyelination was seen in the target area of rat brain one (A, B) and two (C, D) months after sonication. Original magnification, ×20.

Figure 4. Selective antitumor effect of sonodynamic therapy on rat C6 intracerebral gliomas. Hematoxylin and eosin staining of paraffin-embedded sections from irradiated rat brain that had been inoculated with 5×10⁶ C6 glioma cells. Intracerebral tumors grew in the caudo-putamen in sham-operated rat at 2 weeks after the inoculation of tumor cells (A). Selective inhibition of tumor growth while sparing of normal brain tissues was observed with ultrasound alone at 25 W/cm² at 1 MHz for 5 min (B) and with sonodynamic therapy consisting of ultrasound and Rose Bengal (50 mg/kg of body weight) (C). Original magnification, ×20.
Selective antitumor effect of sonodynamic therapy on rat C6 intracerebral tumors. Ultrasound exposure with 25 W/cm² at 1 MHz for 5 min was selected for tumor treatment because these conditions for ultrasound treatment were considered to be safe for normal rat brain tissue. When 5×10⁶ C6 tumor cells were implanted 5 mm deep from the dural surface, intracerebral tumors grew in the caudoputamen in two weeks (Figure 4A). Selective inhibition of tumor growth while sparing normal brain tissues was observed in the groups treated with ultrasound alone (Figure 4B), and these treated with sonodynamic therapy consisting of ultrasound and Rose Bengal (Figure 4C). The largest tumor areas in sham-operated rats and in rats that received sonodynamic therapy without and with Rose Bengal at an intensity of 25 W/cm² for 5 min were 19.53±3.89, 10.64±2.21 and 3.01±1.74 mm², respectively. The tumor area was significantly smaller with ultrasound therapy than in control non-treated animals when rats were treated by ultrasound without Rose Bengal (p<0.002) and with 50 mg/kg Rose Bengal (p<0.001) (Figure 5). There was also a significant difference in ultrasound effect with and without Rose Bengal (p<0.001). There was no significant temperature change in tumor tissue during sonication with 25 W/cm² at 1 MHz regardless of Rose Bengal pretreatment; what change was the same in normal brain tissues (data not shown). These results suggest that the mechanism of the effect of sonodynamic therapy consisting of focused ultrasound and a photosensitizing agent (Rose Bengal) for intracranial experimental gliomas includes a non-thermal cavitation effect under these ultrasound conditions (25 W/cm² at 1 MHz for 5 min), which does not cause any damage in normal brain tissues.

Discussion

In this study, we found that a selective antitumor effect against cerebral glioma while sparing normal brain tissues can be achieved with sonodynamic focused therapy consisting of focused ultrasound and a photosensitizing agent (Rose Bengal). This selective inhibition of tumor growth occurred at 25 W/cm² at 1 MHz for 5 min, which did not harm normal brain tissues. This antitumor effect was significantly enhanced by the prior administration of Rose Bengal at a dose of 50 mg/kg. The ultrasound transducer array probe used in this study had a tight focus at a depth of about 4 mm, as demonstrated previously (22). The incidence of lesions increased with the intensity and duration of exposure. Total irradiation was increased in a stepwise manner by altering the irradiation intensity and/or duration. There was no chemical or heat damage in normal brain regardless of the exposure duration in both the acute and chronic stages with or without the prior administration of Rose Bengal at 25 W/cm² at 1 MHz. When the peak focus intensity was increased to 110 W/cm² at 1 MHz with more than 3 min of exposure, a lesion of coagulation necrosis was seen in the caudate putamen in all of the rats regardless of the use of Rose Bengal. Thus, we determined that exposure at 25 W/cm² at 1 MHz for 5 min was safe for normal rat brain and used these conditions for the treatment of experimental intracranial malignant glioma in rats. Our results showed that focused ultrasound with the photosensitizer Rose Bengal selectively killed tumor without damaging intervening or surrounding normal brain tissues in rats. This is the first report to demonstrate the usefulness of focused ultrasound ablation combined with photosensitizer for the treatment of malignant glioma, although focused ultrasound has been used for the selective destruction of brain tissue in neurosurgical research since the 1940s (17-19), and has been investigated extensively (31-34). The infiltrative tumor cells in surrounding normal brain tissues are good targets for sonodynamic therapy that combines focused ultrasound and photosensitizer. In this study, the activation of the photosensitizer Rose Bengal by ultrasound significantly enhanced the antitumor effect, without the risk of damaging normal intervening brain tissues. Rose Bengal is a xanthene dye that cannot cross the cellular membrane or blood brain barrier. Thus, the enhancement of the ultrasound effect in glioma tissue might be caused by a difference in sensitivity to the ultrasound between normal brain tissue and malignant glioma tissue. A series of investigations appear to show that the responses of malignant cells to ultrasound are not identical to those of normal cells and that cancer cells are more prone to being killed by ultrasound (25). Since blood vessels are very rich in malignant gliomas due to angiogenesis (35), intravascular Rose Bengal might accelerate the ultrasound effect (36). The photosensitizer hematoporphyrin derivative (HpD) has been shown to be selectively localized into all grades of glioma (4), while 5-aminolevulinic acid (5-ALA) is specifically taken up by cancer cells and converted to photosensitizing concentrations of protoporphyrin IX (PpIX)

Figure 5. Areas of tumors 1 week after ultrasound irradiation. The largest tumor areas were measured using NIH image (n=5 in each group). Mean±SD.
We are now working on an experiment on focused ultrasound with Hpd and 5-ALA for the treatment of rat intracranial glioma to achieve a more selective antitumor effect. It also remains to be elucidated whether sonodynamic therapy improves the long-term survival of experimental glioma in rat. Ultrasound energy has been extensively investigated and used over the past three decades in a wide range of clinical procedures (16). Sonication with high intensity focused ultrasound is an effective local cancer treatment that induces cytotoxicity through thermal effects and non-thermal cavitation which generates intracellular reactive oxygen species (20, 24, 38). Ultrasonically induced cavitation is the primary cause of sonoluminescence and sonochemical reactions (39) and, if it can be controlled, may have the greatest potential for therapeutic applications among the nonthermal effects of ultrasound. Non-thermal sonolytic effectiveness of a given low-level ultrasound exposure has been correlated with the generation of acoustic cavitation (40, 41). Ultrasonically induced cavitation, defined as generation and oscillation of gas bubbles, may cause irreversible cell damage and modify the membrane structure and functional properties of the cells to induce cell killing by cell lysis, necrosis or apoptosis (38, 42-44). Sonodynamic therapy is an effective local cancer treatment that induces cytotoxicity through thermal effects and non-thermal cavitation which generates intracellular reactive oxygen species causing oxidative damage to a variety of cellular targets and subsequent tumor necrosis (20). Studies of the in vivo effects of ultrasound in animal brains have shown both thermal and cavitation mechanisms that depend on the applied intensity, ultrasound frequency and duration of exposure (39). The effects of cavitation strongly depend on the tissue type and location. Furthermore, hemorrhage and blood vessel damage could occur when cavitation is present (17, 45).

The present study showed that the strong ultrasound intensity, more than 110 W/cm² at 1 MHz produced coagulation necrosis in normal brain not only by thermal effects but also by a non-thermal effect. The primary mechanism of the forming of such lesions might be considered to be thermal effects due to the dramatic rise in brain temperature after ultrasound exposure in strong focused ultrasound. A non-thermal effect was achieved by the prior administration of Rose Bengal which increased the volume of coagulation necrosis without significantly affecting the temperature rise in normal brain tissue. A selective antitumor effect against cerebral glioma was achieved by weaker focused ultrasound intensity at 25 W/cm² at 1 MHz for 5 min, which did not cause any temperature rise or any damage to normal brain tissue. These results suggest that the mechanism of the effect of the sonodynamic therapy consists of focused ultrasound and a photosensitizing agent (Rose Bengal) for intracranial experimental gliomas includes a non-thermal cavitation effect. In addition, hemorrhage in the lesion was markedly enhanced in animals that had received Rose Bengal. Increased hemorrhage in the lesion may also support the presence of a cavitation effect. Saniabadi et al. reported that arterial thrombosis was initiated by a photochemical reaction in combination with Rose Bengal in vivo (46). The photochemically induced endothelial injury in their model was explained in terms of mechanisms involving reactive oxygen species (28). Therefore, hemorrhage may be the result of vascular injury initiated by a sonodynamic response.

Conclusion

Our results suggest that focused ultrasound with a photosensitizer is effective for the treatment of intracranial malignant glioma in vivo. A selective antitumor effect was produced by non-thermal effect with weaker focused ultrasound intensity at 25 W/cm² at 1 MHz for 5 min which did not cause any damage to surrounding normal brain tissue. This could shed new light on how to prevent local tumor recurrence of malignant glioma, which might improve the treatment outcome in glioblastoma.

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