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Review

Low-intensity ultrasound: A novel technique for adjuvant treatment of gliomas

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ABSTRACT

Glioma is the most common primary malignant tumor of the central nervous system. Although surgical treatment combined with radiotherapy, chemotherapy, and immunotherapy are commonly used for glioma treatment, the prognosis of glioma is still unsatisfactory. The poor effect of glioma treatment could be due to the blocking effect of blood-brain barrier (BBB) on most drugs and the multidrug resistance in tumor cells. In recent years, preclinical trials have shown that low-intensity ultrasound (LIUS) can reversibly open the BBB, inhibit the proliferation of tumor cells, and improve the delivery of drugs to brain tissue. This technology has also recently been used in clinical trials, and achieved encouraging preliminary results. In this review, the existing research results, the effect of LIUS on the adjuvant therapy of glioma under safe conditions, and the physical and biological mechanisms have been discussed. This review aims to show the potential and prospect of LIUS technique in the clinical treatment of glioma.

1. Introduction

Glioma, as the most common primary malignant tumor of the central nervous system [1]. Glioblastoma (GBM) is the most common glioma. Its invasiveness and recurrence rate are significantly higher than other intracranial tumors. Presently, surgery is the first choice for GBM treatment, and it can also be combined with radiotherapy and chemotherapy as comprehensive treatment [2,3]. However, it is difficult to completely remove the tumor due to the invasive and diffuse nature of GBM. The blood-brain barrier (BBB) blocks most therapeutic drugs from reaching the brain. Besides, the special anatomical location of the brain brings about high risks of side effects of radiation therapy of glioma. As a result, the prognosis of GBM is still poor, with a 5-year survival rate of only about 5 % in adults [4]. Therefore, it is necessary to find a treatment that can increase drug concentrations in the brain tissue, inhibit GBM safely and effectively.

Ultrasound, sound wave with a frequency higher than 20 kHz, has been widely used as a diagnostic tool to diagnose diseases in various organs. As a form of energy, ultrasound has also been used in surgery and the treatment of many diseases for decades. Its biological effect can be divided into thermal and non-thermal effects. Intensity is a key parameter of ultrasound. In the past, ultrasound therapy mainly focused on the thermal effect produced by high-intensity ultrasound (100–10000 W/cm^2), which caused tissue damage by selectively raising the temperature of a specific target [5,6]. However, there are many adverse events and defects associated with clinical GBM treatment based on the thermal effect of high-intensity ultrasound due to the nature of brain tissue [7–9]. Compared to high-intensity ultrasound, low-intensity ultrasound (LIUS, less than 3 W/cm^2) is an output in low-intensity pulse wave mode, which has the least thermal effect while maintaining the transmission of sound energy to the target tissue, and provides non-invasive physical stimulation for disease treatment. LIUS treatment mainly uses its non-thermal effect, including cavitation and other "mechanical effects", such as acoustic microstreaming and acoustic radiation forces [10–12].

This review summarizes the preclinical and clinical research progress

Abbreviations: BBB, Blood-brain barrier; LIUS, Low-intensity ultrasound; GBM, Glioblastoma; UTMC, Ultrasound-targeted microbubble cavitation; MI, Mechanical index; PRF, Pulse repetition frequency; TRPV4, Transient receptor potential vanilloid 4; BTB, Blood-tumor barrier; EMAP-II, Endothelial monocyte activating polypeptide II; GMECs, Glioma microvascular endothelial cells; TMZ, Temozolomide; MGMT, O^6-methylguanine-DNA methyltransferase; UTMD, Ultrasound-targeted microbubble destruction; NO, Nitric Oxide; PFS, Progression-free survival.

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of LIUS in treating gliomas in recent years. The differences in various acoustic parameters and the possible mechanism of LIUS in the treatment of glioma have also been discussed for further clinical application.

2. Acoustic principle of therapeutic effect of LIUS

The non-thermal effects of LIUS are thought to be mainly mediated by cavitation (the activity of bubbles produced by submicron airbags (cavitation nuclei) in the ultrasonic field). The high compressibility and acoustic impedance of microbubbles cause periodic oscillation (stable cavitation) or severe collapse (inertial cavitation) during the positive and negative pressure oscillation of ultrasound [13]. The repeated contraction and expansion of the microbubbles under the action of ultrasound causes the liquid to flow around the microbubbles at stable cavitation, while the structure of the microbubbles is intact. The microflow exerts a shear force on the cell, resulting in the transient permeability of the cell membrane [14,15]. Excessive ultrasonic pressure in inertial cavitation leads to microbubble collapse, and results in strong mechanical stress, shock wave, and microjet, as well as free radicals and high local temperature, leading to irreversible cell or tissue damage [16,17]. The use of LIUS to induce cavitation of microbubbles in the irradiated area to produce biological responses in tissues or cells is called ultrasound-targeted microbubble cavitation (UTMC).

However, LIUS can also induce biological effects in the absence of microbubbles [18–20]. Krasovitski established a cell-level bilayer membrane model, combined with bubble dynamics and cell biomechanics, to determine the dynamic behavior of two lipid bilayer lobules. It is found that under appropriate conditions, oscillatory sound waves at millimeter wavelengths can be converted into intracellular deformations at nanometer and micron levels. This cyclic expansion and contraction can stimulate the stretch and release cycle in the cell membrane and cytoskeleton, and thus activate mechanically sensitive proteins and increase membrane permeability. There is no need for microbubbles in the tissue since the cellular bilayer membrane structure can directly convert sound energy into mechanical stress and strain at the subcellular and cellular levels. However, exogenous bubbles can enhance this mechanical effect [21].

The biological effect of LIUS is associated with the acoustic intensity, amplitude (acoustic pressure), and frequency of the ultrasonic source. The acoustic intensity is the acoustic energy of the ultrasonic beam passing through the unit cross-sectional area in unit time. It is proportional to the square of frequency and the square of acoustic pressure. The mechanical index (MI) is a semi-quantitative index used to describe the biological effect of ultrasound, which is a suitable index for the expression of ultrasonic mechanical damage. FDA made it a requirement for ultrasound manufacturers to display real-time MI data on the display screen of ultrasonic instruments. MI is proportional to the acoustic pressure and inversely proportional to the square root of the frequency ($MI = P/\sqrt{f}$). The higher the acoustic pressure, the lower the frequency and the higher the MI, and thus the easier it is to produce mechanical action. Therefore, selection of appropriate intensity, pressure and frequency is essential for generating ultrasonic cavitation and other mechanical actions based on acoustic theory. However, there are still many acoustic parameters that affect the mechanical action of ultrasound in the practical application of ultrasonic instruments.

3. Ultrasonic parameters and safety of LIUS in opening BBB

The BBB acts as a permeability barrier between capillaries and brain tissue. It is connected by a junction complex composed of endothelial cells through tight junctions and adhesive junctions, which controls and restricts the entry of vascular lumen molecules into the brain through paracellular or transcellular pathways [22]. Only small fat-soluble molecules with molecular weight of no more than 500 Da can pass through the BBB [23]. BBB inhibits drug treatment for central nervous system diseases since only 5 % of about 7000 known potential therapeutic drugs can pass through the BBB [24–26]. Therefore, it is important to find ways of regulating or bypassing the BBB to enhance the efficacy of glioma drugs [27]. The LIUS technology combined with intravenous injection of microbubbles is used to induce the opening of the BBB, and has been studied for many years. The method mainly uses the cavitation effect to target and instantaneously and reversibly open the BBB (back to normal after 6–24 h) [28], increasing the permeability of some drugs that cannot easily pass through the BBB and enhancing drug efficacy.

Some studies have analyzed the open effect of LIUS on BBB using the Evans blue injection or MRI-enhanced scanning imaging in animal models. The relationship between the opening degree of ultrasound to the BBB and various acoustic parameters, including acoustic pressure, acoustic intensity, frequency, pulse duration, pulse repetition frequency (PRF), total irradiation time, type and dose of microbubbles injected, has also been analyzed. Most studies have shown that the degree of BBB opening increases with the increase of acoustic pressure. Therefore, the ultrasound-induced opening of the BBB may be controlled by regulating the acoustic pressure. Hynynen, McDannold and Vkhodtseva found that the BBB opening rates for irradiated rabbits after craniotomy were 50 % and 100 % at acoustic pressures of 0.4 MPa and 0.8 MPa, respectively [29]. Red blood cell extravasation occurs in the surrounding brain tissue when the acoustic pressure exceeds 0.5–0.6 MPa. Besides, brain tissue necrosis and apoptosis may occur when the acoustic pressure is above 2.3 MPa [30]. Various ultrasonic frequencies (28 kHz-8 MHz) have been used to evaluate and verify the BBB opening rates. Most studies have indicated that under the consistent acoustic pressure, lower frequency is associated with less tissue attenuation, higher MI, and more significant BBB opening effect, but accompanied by higher risks of inertial cavitation and brain tissue damage. Under the condition of keeping the BBB open, the relatively high ultrasonic frequency can make the focus of the beam more concentrated, which causes less tissue damage to the non-target area and is safer [31]. The use of lower frequency is associated with a larger focus area, and thus may not be suitable for small lesions that need to be accurately located, but it may be suitable for the treatment of large lesions. However, some experiments have found that at about 2 MHz frequency, brain tissue damage is more likely to occur compared with other low frequency ultrasound. The threshold of BBB opening at this frequency is closer to the microbubble inertia cavitation threshold, which causes damage [32]. Presently, the effective recommended ultrasonic frequency range is between 200 kHz and 1.5 MHz [33].

The relationship between other parameters and BBB opening has also been assessed. Some studies have shown that microbubble type, microbubble dose, ultrasound irradiation time and PRF affect the opening of BBB. Moreover, excessive increase in these parameters does not cause brain tissue damage [31]. Some other studies have found that the degree of BBB opening is not significantly different when changing the dose of PRF or microbubble. The pulse duration determines the degree of BBB opening caused by ultrasound and the acoustic pressure threshold (when the BBB opening rate is up to 50 %) [34]. Too short pulse duration (10 μs) results in insufficient time for the biological action of ultrasound to cause BBB opening [35]. The BBB opening increases when the pulse duration increases from 0.1 ms to 10 ms. Pulse duration of more than 10 ms cannot further increase the degree of BBB opening, which is possibly because the microbubbles in the irradiated area have already been completely destroyed. Although the 100 ms pulse duration has not been found to cause obvious damage to the brain tissue, for maximum biological safety, most preclinical studies used the 10 ms pulse duration as the irradiation condition. The current commercial ultrasound contrast agents can open the BBB with only a slight difference, which may be due to the composition of the contrast agent and the size of microbubbles [29]. Some studies have used diagnostic ultrasound equipment to irradiate outside the rat skull. Diagnostic ultrasound combined with self-made contrast agent Zhifuxian after

Table 1
Summary of preclinical studies on BBB opening by LIUS.

Animal model	Irradiation method	US device	Main acoustic parameters					Dose of microbubbles	References
			Acoustic pressure	Frequency	PRF	Pulse duration	Irradiation time		
Mouse	Extracranial irradiation	SonoCloud(CarThera)	0.3 MPa	1 MHz	1 Hz	NA	120 s	Lumason (7.5 ml/kg)	. Sonabend et al[59]
Mouse	Extracranial irradiation	SonoCloud(CarThera)	0.3 MPa	1 MHz	1 Hz	NA	120 s	Lumason (200 μl)	. Heimberger et al [64]
Mouse	Extracranial irradiation	Sonic Concepts	0.3 MPa	500 kHz	1 Hz	10 ms	60 s	SonoVue (0.1 ml/kg)	. Liu and Wei et al [55]
Mouse	Extracranial irradiation	Imasonics, Besancon	0.28–0.55 MPa	NA	1 Hz	10 ms	180 s	BG8235 (Bracco Suisse, 50 μl)	. Roth and Leroux et al[58]
Mouse	Extracranial irradiation	Self-development	0.64 MPa	1.1 MHz	1 Hz	10 ms	60 s	Self-development	. Chen and Yan et al [60]
Mouse	Extracranial irradiation	Unknown	0.3 MPa	1.05 MHz	1 Hz	23.8 ms	120 s	Sonovue (200 μl)	. Idbaih et al[61]
Rat	Extracranial irradiation	Self-development	0.8 MPa *	1.5 MHz, 1.7 MHz	1 Hz	10 ms	30 s	Optison (0.1 ml/kg)	. Treat et al[38]
Rat	Extracranial irradiation	Imasonics, Besancon	0.6 MPa *	500 kHz	1 Hz	10 ms	60 s	SonoVue (0.1 ml/kg)	. Wei et al[54]
Rat	Extracranial irradiation	Self-development	0.68–0.72 MPa	690 kHz	1 Hz	10 ms	60 s	Definity (10 μl/kg)	. Aryal et al[53]
Rat	Extracranial irradiation	Sonic Concepts	0.36–0.7 MPa	0.5 MHz	1 Hz	100 ms	90 s	Optison (100 μl/kg)	. Liu et al[65]
Rat	Extracranial irradiation	Self-development	0.3, 0.6 and 1.5 MPa	515 kHz, 1.6 MHz	1, 2 and 5 Hz	1, 10 and 100 ms	30, 60, 120 and 300 s	SonoVue (30 μl/kg) or Definity (20 μl/kg,100 μl/kg)	. Shin et al[30]
Rat	Extracranial irradiation	GE Vivid 7 (M4S)	1.82 MPa	1.9 MHz	2567 Hz	0.84 ms	600 s	zhifuxian	. Liu et al[37]
Rat	Extracranial irradiation	GE Vivid 7 (M4S)	1.82 MPa	1.9 MHz	2567 Hz	0.84 ms	600 s	zhifuxian	. Xu et al[36]
Rat	Intracranial irradiation	Self-development	0.071–0.25Mpa	1.15–1.2 MHz	1 Hz	10 ms	120 s	Definity (20 μl/kg)	. Hynynen et al[45]
Rabbit	Extracranial irradiation	Self-development	0.4 MPa	0.69 MHz	1 Hz	10 ms	20 s	Optison (50 μl/kg)	. Hynynen et al[29]
Rabbit	Intracranial irradiation	Self-development	0.2, 0.4, 0.5, 0.8, 1.1 and 1.5 MPa	690 kHz	1 Hz	10 ms	NA	Definity (10 μl/kg) or Optison (50 μl/kg)	. McDannold et al [31]
Rabbit	Intracranial irradiation	Self-development	0.4–1.5 MPa	690 kHz	0.5, 1, 2, 5 Hz	0.1, 1, 10 ms	20 s	Optison (50,100,250 μl/kg)	. McDannold et al [34]
Rabbit	Intracranial irradiation	Self-development	0.3–2.3 MPa	2.04 MHz	1 Hz	10 ms	20 s	Optison (50 μl/kg)	. McDannold et al [32]
Macaques	Extracranial irradiation	Sonic Concepts	0.2–0.4 MPa	0.5 MHz	2 Hz	10 ms	120 s	Self-development	. Downs et al[43]
Rhesus macaques	Extracranial irradiation	ExAblate 4000, TcMRgFUS (InSightec)	0.149–0.7 MPa	220 kHz	1.1–0.28 Hz	10 ms	70,150 s	Definity (10 μl/kg,20 μl/kg)	. McDannold et al [44]

1. * In this study, the actual acoustic pressure at the focal point has been predicted after the wave through the skull.

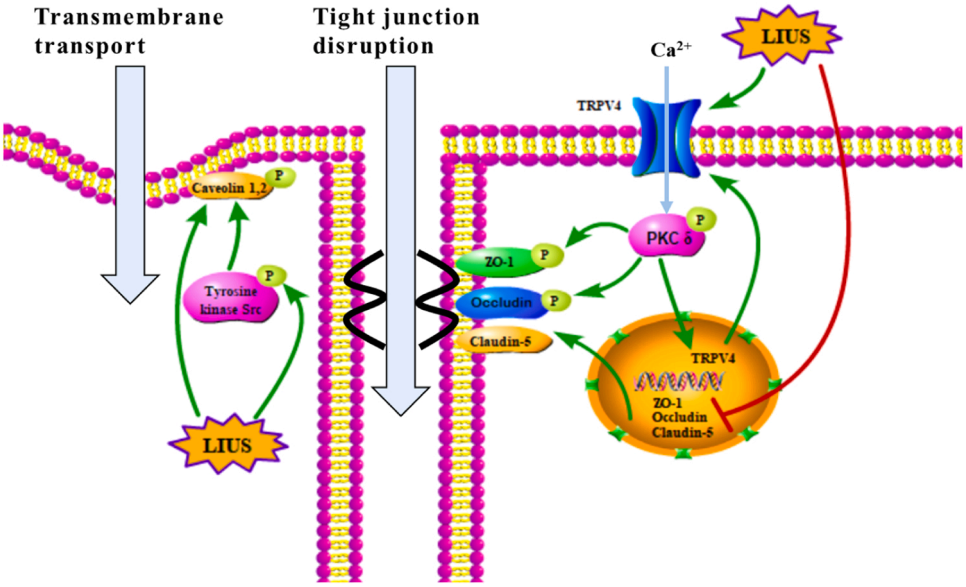

Fig. 1. The biological mechanism of LIUS to open the BBB. The main biological mechanism is thought to be the disruption of tight junctions and the enhancement of cell transmembrane transport.

conversion under the target area (about 0.24 MPa pressure, MI1.3, irradiation; 10 min) can open the BBB [36,37]. However, at present, most experiments have used therapeutic ultrasound instruments to open BBB. It is more difficult to directionally open the BBB using diagnostic ultrasound than using therapeutic ultrasound due to the non-focusing nature of diagnostic ultrasound beam.

One hindering factor for the application of transcranial LIUS is the nature of skull. Its high attenuation and non-uniform composition result in significant loss of acoustic energy and phase-frequency distortion. As a result, the amplitude, frequency, and spatial properties of the transmitted acoustic waves are affected. Therefore, different experimental animals, or even different anatomical regions of the same subject, may have slightly different ultrasound parameters for BBB opening. For instance, Treat et al. found that the threshold of ultrasound intensity causing BBB opening in the posterior quadrant (such as thalamus, hippocampus, and superior colliculus) was lower than that in the anterior quadrant of the brain (such as caudate putamen in transcranially irradiated rats) [38]. Some studies have used targeted ultrasound system to detect the ultrasonic attenuation of rat skulls and found that the acoustic pressure attenuation of skulls is about 61 % at 1.5 MHz. The safe and most appropriate parameters for opening BBB in rats using LIUS combined with microbubbles are focal point pressure of 0.4 MPa, pulse duration of 3 ms, and irradiation time of 1 min. Researchers have also suggested that the focal position pressure should be predicted and a personalized treatment plan should be made according to the skull anatomy and thickness before transcranial application of LIUS to improve the clinical efficiency and safety of LIUS for BBB opening [39].

Inflammation is one of the main side effects of BBB opening on normal brain tissue. Astrocyte activation can induce inflammatory response [40,41]. A study showed that microbubbles and feedback control of acoustic pressure levels could simultaneously achieve LIUS-mediated BBB disruption and reduced inflammatory responses [42]. Besides, repeated long-term opening of BBB in the caudate and putamen regions of non-human primates was not associated with any significant side effects based on the vital signs, cognitive behavior and MRI. However, histological changes were not assessed in this study [43]. McDannold analyzed the safety of ultrasound irradiation instruments and parameters designed for human clinical application at the level of rhesus monkeys, and found no significant side effects on cognitive behavior, imaging performance and histology [44]. With the increase of the degree of ultrasound opening BBB, the risk of tissue injury also increases. In the formulation of LIUS irradiation parameters, the first factor to consider is the disease and purpose of opening BBB treatment. If the purpose of BBB opening is to assist in delivery of chemotherapeutic drugs to glioma lesions, the brain damage induced at the core or acoustic edge of the tumor is less important, because it is designed to help kill tumor cells and slow cancer progression. In contrast, if the purpose of treatment is to assist in drug delivery therapy to restore or preserve brain function, more attention must be paid to the brain damage caused by LIUS and the side effects caused by opening BBB. Table 1.

4. The biological mechanism of LIUS to open the BBB

Some scholars have studied the biological mechanism of BBB opening caused by LIUS. Hynynen et al. assessed the relationship between the destruction of capillaries and acoustic pressure in rat brain tissue at the subcellular level using two-photon fluorescence microscopy. They found that there was no contraction or dilation of blood vessels when the acoustic pressure was below 0.1 MPa. However, a slow increase of capillary wall infiltration occurred [45], which indicated that BBB opening was not only due to the physical effect of microbubbles on the vessel wall. The main biological mechanism is thought to be the disruption of tight junctions and the enhancement of cell transmembrane transport. Chen et al. found that LIUS could stimulate the activation of transient receptor potential vanilloid 4 (TRPV4) channels, promote calcium influx, induce PKC-δ activation, and increase tyrosine phosphorylation of tight junction related proteins ZO-1 and occludin even in the absence of microbubbles, leading to the dissociation of these tight junction proteins and BBB opening. TRPV4 agonist can enhance the opening effect of LIUS. Chen's study has shown that opening BBB without adding microbubbles has certain advantages in clinical application. For instance, it can avoid side effects of microbubbles, reduce vascular damage caused by cavitation and reduce cost [46].

Blood-tumor barrier (BTB) occurs when BBB is destroyed during tumor progression. Although BTB has stronger permeability than BBB, it has the characteristics of BBB and thus limits the penetration of therapeutic drugs. LIUS combined with microbubbles can down-regulate the mRNA and protein expressions of tight junction-related proteins (ZO-1, occludin, and claudin-5) in C6 rat gliomas in vivo and in vitro. The expressions can reach maximum after 2–3 h of treatment and gradually return to normal after 12–24 h. LIUS irradiation can improve the permeability of BTB by opening the paracellular pathway [47,48],

which can be enhanced by combined with low-dose bradykinin or endothelial monocyte activating polypeptide II (EMAP-II) [49,50]. Besides the paracellular pathway, transcellular pathway has also been found to contribute to BTB permeability change induced by LIUS. LIUS combined with microbubbles increases BTB permeability by promoting pinocytosis of glioma microvascular endothelial cells (GMECs) of rat C6 glioma BTB model and increasing the expression of caveolin-1 and caveolin-2. The activation of tyrosine kinase Src, caveolin-1 and caveolin-2 promotes LIUS-induced caveolae-mediated endocytosis [51] (Fig. 1). The combination of LIUS and low-dose bradykinin can also enhance transcellular transport [52]. The study of the biological mechanism of LIUS opening the BBB provides a theoretical basis for the combined application of LIUS and BBB-opening drugs. Compared with single action, the combination can be more efficient, limited and safe to open the BBB. Therefore, more in-depth exploration of the biological mechanism and target of LIUS opening BBB is of great significance for the cross-BBB drug use of glioma.

5. LIUS improves drug delivery in glioma by opening BBB

Many studies have found that LIUS combined with microbubbles can enhance the delivery of chemotherapeutic drugs to brain tissue or tumor tissue by opening the BBB, increasing the drug concentration, and thus achieving therapeutic purposes. The method can also increase the distribution of doxorubicin in brain tissue [53], which is linearly enhanced with the dose of microbubbles [38]. Moreover, it can increase the local content of temozolomide (TMZ), a first-line drug for GBM [54,55]. Drug resistance of glioma to TMZ is related to the expression of O^6-methylguanine-DNA methyltransferase (MGMT) gene, which can repair the cytotoxic damage of O6-methylguanine (O6-Meg) induced by TMZ and cause drug resistance [56,57]. Roth et al. prepared liposomes loaded with small molecular MGMT inactivating agents, and then mediated BBB opening using LIUS. They found that the intracranial concentration of increased, tumor growth decreased, the systemic toxicity of inactivating agents was overcome and the survival time of glioma-bearing mice was significantly prolonged [58]. An intracranial experimental study of nude mice, in which transcranial LIUS was treated with 1 MHz and 0.3 MPa alone for 120 s, showed that LIUS could locally destroy the BBB and increase the content of paclitaxel in brain tissue to inhibit glioma based on NaFL fluorescence imaging [59]. LIUS alone cannot significantly affect the survival time of tumor-bearing mice under these conditions. Another study treated glioma with paclitaxel liposome and showed that LIUS had a non-invasive and efficient mode of transport, which improved the effect of chemotherapy on glioma [60]. LIUS combined with microbubbles can also increase the infiltration of carboplatin in brain tissue and improve the survival rate of nude mice [61].

Presently, immunotherapy, including immune checkpoint inhibitors, immunomodulators and cell therapy, cannot be fully used for the treatment of gliomas because of the existence of BBB. LIUS can promote the entry of antibodies and immune cells into the glioma microenvironment [62,63]. In one study, in which glioma was treated with anti-PD-1 alone, only a low level of antibody infiltration in the brain tissue and a slight enhancement of focal delivery at the potential site of tumor implantation were observed. The destruction of BBB caused by the combination of LIUS and microbubble can enhance the delivery of anti-PD-1 antibody in the irradiated brain tissue. LIUS can also assist in transporting CXCL10-expressing APC to the glioma microenvironment for deposition, and activate T cells in the tumor microenvironment which directly kill tumor cells. Its effect is better than that of direct intraperitoneal injection [64]. LIUS can increase IL12 content in tumor tissue, increase the ratio of CTL and CTL/Treg, and inhibit the growth of glioma cells after intraperitoneal injection of IL-12, without enhancing the immune response of systemic or normal tissues [65]. Another study found that LIUS could enhance the delivery of anti-angiogenic monoclonal antibody bevacizumab to brain tissue, and thus exert the anti-GBM effect [66].

LIUS combined with microbubbles can destroy the BBB, and facilitate the delivery of drugs to brain tissue. This transfer of substances between brain and blood may also be bidirectional. Chen et al. tested and verified that LIUS combined with microbubbles could safely mediate the release of brain tissue-specific biomarkers from the brain into the peripheral circulation in mice and pigs. These results show that peripheral liquid biopsy technology can be used to enhance glioma treatment [67,68].

In the past decade, ultrasound opening BBB has improved drug delivery, and the method has developed from ultrasound irradiation combined with intravenous drug injection, to carrying therapeutic drugs or genes into microbubble shell or gas core through physical and chemical actions, with ultrasound-induced microbubble release in the target area, which has greatly improved the efficacy of anti-glioma drugs and gene therapy. This technique is called ultrasound-targeted microbubble destruction (UTMD) [69,70]. This method can protect the drug, prolong its circulation half-life and reduce the systemic adverse reactions of the drug [71]. Furthermore, through the preparation of submicron microbubbles to match the resonant frequency with the ultrasonic frequency, the stable cavitation can be maximized and the inertial cavitation can be reduced, and the radiation damage to the surrounding brain tissue can be reduced [72]. The targeted release of such drug-loaded microbubbles is an important direction for future ultrasound therapy research. At present, there have been studies on the drug doxorubicin and carmustine through this method to improve the inhibition of glioma. There are also many drugs that can be tried by this method in the future. For example, high concentrations of Nitric Oxide (NO) can cause tumor cell death [73], which has the potential to treat cancer, but most of its delivery agents have a short half-life and poor targeting. The UTMD may be a method to help solve the problem of NO in the treatment of gliomas. However, at present, most of the studies on the use of UTMD are in the cellular and animal experimental stage, and there are few reports on its application in large animal models, and there are still some problems which need to be solved before clinical trials. For example, how to improve the production process and increase the yield, drug loading and stability of drug-loaded microbubbles. It is necessary to explore ultrasonic parameters suitable for clinical application of microbubble drug release, and even develop more advanced instruments. In conclusion, in order to apply this promising technology to the clinic as soon as possible, multidisciplinary support and cooperation from materials science, chemistry, physics, pharmacology, etc. are needed.

6. Biological effects of LIUS on glioma cells

Some studies have discussed the biological effects of LIUS on tumor cells in recent years due to its therapeutic effect. The biological effects of LIUS, include regulation of cell proliferation and apoptosis, opening membrane channel, etc. Zhang et al. found that LIUS combined with microbubbles could inhibit the proliferation of glioma cells and promote apoptosis of rat glioma C6 cell line in intensity and time-dependent manner with the frequency of 2 MHz, intensity higher than 83.4 mW/cm^2 and the irradiation time of more than 30 s. Moreover, the method did not affect normal astrocytes. However, it produced apoptosis effect on rat astrocytes when the intensity was 290 mW/cm^2 and the irradiation time was more than 30 s. In addition, LIUS could reduce the mRNA levels of multidrug resistance proteins P-gp and MRP1 in glioma cells and increase the sensitivity to doxorubicin. LIUS combined with doxorubicin can down-regulate the expression of PI3K/Akt/NF-kB-related proteins [74]. Some studies have shown that ultrasound-assisted curcumin can inhibit the activation of VEGF/PI3K/Akt and down-regulate MRP1, leading to the apoptosis of C6, U87 and U251 at low concentration and enhancing the sensitivity of the drug in vivo or in vitro [75–77]. Hayashi et al. found that LIUS could inhibit the proliferation of glioma U251 and U105MG after irradiation for 30 s (at the intensity of 300 mW/cm^2) in vitro. Observation under the electron microscope

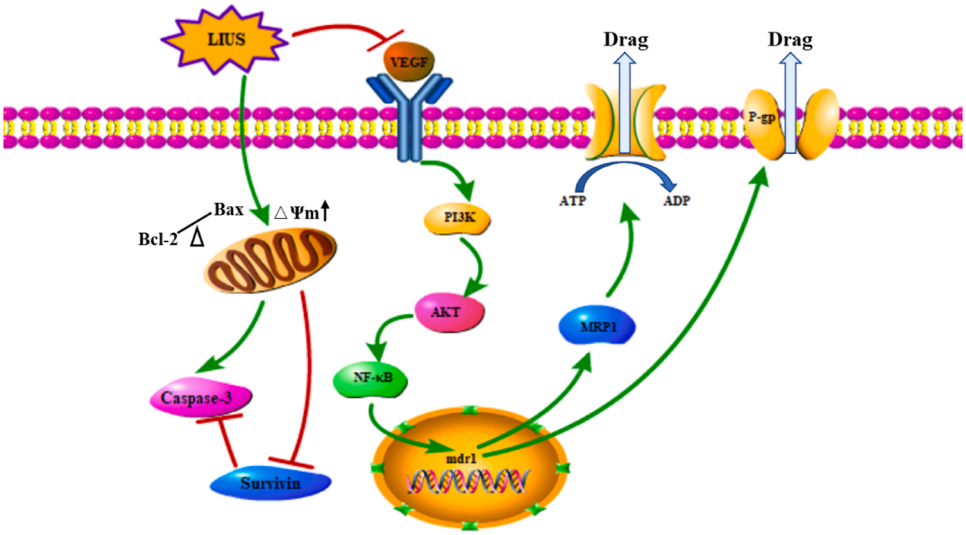

Fig. 2. Biological effects of LIUS on glioma cells. The studies on the effect of LIUS on glioma cells found that under specific acoustic parameters, it could cause glioma cells apoptosis and increase the efficacy of anti-glioma drugs.

showed that the surface structure of the cell membrane was slightly damaged, and the microvilli disappeared [78].

Many studies have analyzed the mechanism of ultrasound-mediated cell membrane disturbance and showed that the permeability of the membrane was reversible within 24 h [79–81]. It was found in glioma C6 cell line in vitro that ultrasound could disturb the cell membrane and increase the permeability (which lasted as long as 24 h) in the presence of microbubbles, but it did not affect cell vitality. Moreover, although the transient loss of cell membrane asymmetry may lead to phosphatidyl serine exposure in the outer lobules of the cell membrane, it does not cause apoptosis. Additionally, ultrasound treatment can disturb the membrane structure that has not come into contact with microbubbles after 24 h. The membrane potential of mitochondria slightly reduced, and returned to normal or even increased after some time[82](Fig. 2).

Compared with the study in which LIUS opens BBB and increases the concentration of drugs in brain tissue to assist in the treatment of gliomas, the research on the direct inhibitory effect of LIUS on glioma cells is still in its infancy. The pathways and targets involved in tumor cell therapy are very complex, and the research is more challenging than the research on opening BBB. The present researches are not sufficient to explain the biological effects of LIUS on glioma cells. Therefore, the potential cellular biological mechanism needs to be further studied.

7. Clinical study of LIUS in the treatment of glioma

Most clinical trials of LIUS for the treatment of gliomas that can be obtained through ClinicalTrials.gov mainly focused on BBB opening, increasing drug concentration in brain tissue, and synergistic treatment. In summary, the studies show that LIUS can play a positive role in the treatment of glioma in clinical application. TMZ is the first-line drug for the clinical treatment of glioma and can cross the BBB, but the concentration of TMZ in brain tumor tissue is only about 20% of that in plasma [83]. TMZ concentration may only rise to 35% of the plasma level even if simultaneous radiotherapy improves BBB permeability [84]. Several single-centers, prospective phase I clinical trials (NCT02343991, NCT03712293, NCT02986932, NCT03616860, NCT03322813) have used transcranial-focused ultrasound equipment consisting of 1024 separate transducers at a frequency of 220 kHz (ExAblate Neuro, InSightec). MRI-guided ultrasound irradiation has also been used during the operation. Moreover, the effect of ultrasound combined with microbubbles on BBB has been assessed using extracranial irradiation. All patients were intravenously injected with chemotherapy drugs one day before ultrasound irradiation, and the opening of the BBB was observed by MRI. It was found that ultrasound could safely and reversibly open the BBB without showing brain hemorrhage or hematoma, and BBB integrity restored after 20 h [85], thus increasing the drug absorption of brain tissue in the irradiated area [86,87]. Moreover, the TMZ concentration could be increased by 7.7 times. The patients with tumor recurrence had an average progression-free survival (PFS) of 13.5 months, while the other patients had a median PFS of at least 15 months, with the one-year overall survival rate of 100 % [88]. Clinical trials found that LIUS irradiation could increase the number of brain-derived biomarkers in peripheral circulation, such as protein, cfDNA, and EVs, which indicates that combination of this technique with liquid biopsy can be used to diagnose and monitor gliomas [89].

Preclinical extracranial irradiation experiments have shown that the skulls of small animal models are relatively thin, making it possible to focus in the brain even with a simple unit of ultrasonic transducers. However, human skull is thicker and absorbs up to 90% of the ultrasonic energy, and large hemispherical phased array equipment with hundreds of separate ultrasound units is required to achieve similar results (ExAblate Neuro, InSightec) [90]. This process requires long-term operation under MRI detection, and is thus inconvenient. French scholars have developed a small implantable ultrasound transducer (SonoCloud, CarThera), which avoids the shortcomings of transcranial ultrasound irradiation. However, the method requires invasive surgery. After the equipment was proved safe and effective in animal experiments, a single-center, prospective study has been conducted (NCT02253212), since 2014 [91–94]. In the study, a device (diameter: 11.5 mm) was implanted in 19 GBM patients at 1 MHz, and the device was activated at least once a month. The experimental design was to study the increasing dose of ultrasonic irradiation, and the acoustic pressure increased gradually from 0.41 MPa to 1.15 MPa. MRI was used to observe the opening ability and safety of ultrasound combined with microbubbles on the BBB. Results showed that BBB was temporarily destroyed after 65 ultrasonic treatments when the acoustic pressure was above 0.8 MPa. BBB destruction increased with the acoustic pressure increasing. Moreover, there was no damage in the surrounding brain tissue even at 1.1 MPa, and the patients had no adverse reactions. Furthermore, patients with disrupted BBB had increased sensitivity to carboplatin therapy and the median PFS increased from 2 to 3 months to 4.11 months, and the overall survival time increased from 6 to 9 months to 12.94 months [95,96].

Extracranial devices and implantable devices each have advantages and disadvantages, and the clinical use of these devices may be complementary. For instance, extracranial equipment is non-invasive and

Table 2
Clinical trials of LIUS in the treatment of glioma, in which the results have been reported.

US device	Irradiation method	Main acoustic parameters						Dose of microbubbles	Drug	NCT No.	Number of cases	PI location	Phase	References
		Acoustic pressure	Acoustic power	Frequency	Pulse repetition frequency	Duty cycle	Irradiation time							
ExAblate Neuro (InSightec Tirat Carmel)	Extracranial irradiation	NA	4–10 W	220 kHz	NA	0.74%	50 s	Definity (4 μl/kg)	5 cases of temozolomide and 1 case of doxorubicin liposome	NCT02343991	5	Canada	I	. Mainprize et al[87]
ExAblate Neuro Model 4000 Type 2.0 (InSightec)	Extracranial irradiation	NA	3–10 W	220 kHz	NA	NA	Average of 80 s, one or two days after chemotherapy	Definity (4 μl/kg)	Temozolomide	NCT03712293	6	Korea	I	. Chang et al [85,86]
ExAblate Neuro Type 2.0 (InSightec)	Extracranial irradiation	NA	3–21 W	230 kHz	NA	8%	NA	Definity (4–5 μl/kg)	No chemotherapy drugs	NCT03322813	4	USA	0	. Woodworth et al[84]
ExAblate Neuro Model 4000 Type 2.0 (InSightec)	Extracranial irradiation	NA	NA	220 kHz	NA	NA	Average of 110 s, 30 min after chemotherapy	Definity	Temozolomide	NCT03616860	9	Canada	I	. Lipsman et al [88]
SonoCloud (CarThera)	Intracranial irradiation	0.41, 0.53, 0.66, 0.78, 0.90, 1.03, 1.15 MPa	NA	1.05 MHz	0.5, 1 Hz	1.2% , 2.4%	150–270 s , once a month	Sonovue (0.1 ml/kg)	Carboplatin	NCT02253212	21	France	I/IIA	. Carpentier et al[94,95]

can focus on deep and variable targets in the brain. However, the operation is complex and takes long time, and thus it is suitable for the treatment of lesions with small target area and deep location. In contrast, the implantable device has a short operating time and a large area of action, and thus it is suitable for the treatment of large, superficial, invasive lesions or those requiring repeated activation and opening of BBB. However, it must be surgically implanted, which is a kind of invasive treatment. At present, the sample size of clinical studies of LIUS in the treatment of glioma is small, and there is no multi-center study with large samples. Due to the limitation of increasing the drug content in brain tissue by opening the BBB, there is no evaluation of the tumor suppressive effect of LIUS alone or combined with microbubbles. The clinical trials of LIUS to enhance glioma immunotherapy and the targeted release of novel ultrasound microbubble-loaded drugs are also worth looking forward to. Table 2.

8. Conclusions and perspectives

Although the data on LIUS in the treatment of various diseases over the past few decades are encouraging, most of its applications have been limited to preclinical studies. Many aspects should be considered for the transition from preclinical research to clinical application. First, optimization of ultrasound parameters is key since the effectiveness of ultrasound therapy depends on multiple ultrasound parameters. This optimization process depends on the biological characteristics of the disease. Therefore, the LIUS parameters used in other sites and disease treatment may not be suitable for glioma treatment based on the anatomical, physiological and morphological properties. Excessive ultrasound irradiation in the central nervous system diseases activates inflammatory changes, which may aggravate brain injury. Although such side effects may be ignored in the treatment of neoplastic lesions, more effective and safer acoustic parameters should be explored. Moreover, due to the significant differences in skull thickness, brain tissue size, and acoustic attenuation between humans and animals, the changes of acoustic parameters in the location of acoustic energy irradiation and the target area of the brain should be assessed to design different irradiation schemes for different tumor locations for better clinical applications.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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