Recent Advances in Research on Vascular Permeability to Establish Novel Therapeutic and Drug Delivery Strategies for Intractable Diseases

Review

Enhanced Vascular Permeability by Microbubbles and Ultrasound in Drug Delivery

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Ultrasound and microbubbles, an ultrasound contrast agent, have recently increased attention to developing novel drug delivery systems. Ultrasound exposure can induce mechanical effects derived from microbubbles behaviors such as an expansion, contraction, and collapse depending on ultrasound conditions. These mechanical effects induce several biological effects, including enhancement of vascular permeability. For drug delivery, one promising approach is enhancing vascular permeability using ultrasound and microbubbles, resulting in improved drug transport to targeted tissues. This approach is applied to several tissues and drugs to cure diseases. This review describes the enhancement of vascular permeability by ultrasound and microbubbles and its therapeutic application, including our recent study. We also discuss the current situation of the field and its potential future perspectives.

Key words blood vessel; brain; cancer; drug delivery; microbubble; ultrasound

1. INTRODUCTION

The drug delivery system is essential to cure diseases with high therapeutic effects and low side effects by improving a distribution of drug in the body. Various drug delivery systems have been studied, such as chemical modification to change physiological characteristics of drug and development of drug loading carriers to control the distribution of drug.¹⁻³⁾ Additionally, physical energies, such as thermal energy, light energy, magnetic force, proton concentration, and ultrasound are used to develop drug delivery systems.⁴⁻⁸⁾ In most of the systems, a drug is systemically administered and distributed *via* blood flow. Then, the drug must pass vascular endothelial cells and act on targeted parenchyma cells except targeting endothelial cells. Therefore, vascular endothelial cells are a major obstacle to achieving an appropriate drug distribution. An increase in vascular permeability is a promising approach to overcome the obstacle. Many strategies have been studied using biologically active substances such as bradykinin and nitric oxide and physical energy such as thermal energy and ultrasound.^{4,8-10)} The combination of ultrasound and microbubbles, an ultrasound contrast agent, has increased attention because it can enhance vascular permeability by mechanical effects of microbubbles. To establish noninvasive and efficient drug delivery systems, research and development using ultrasound and microbubbles have dramatically proceeded.¹¹⁻¹⁴⁾

This review describes the enhancement of vascular permeability by ultrasound and microbubbles and its therapeutic application, including our recent study. We also discuss the current situation of the field and its potential future perspectives.

2. MICROBUBBLES AND ULTRASOUND

Ultrasound is clinically used in ultrasound imaging diagnosis for a long time. Ultrasound and its imaging device have several advantages such as non-invasiveness, non-radioactivity, superior portability, and low cost. Recently, ultrasound is also used for therapy. A high-intensity focused ultrasound, which can concentrate ultrasound energy in small point and ablate tissue, has been approved in Japan to treat essential tremors and Parkinson disease. It is also tried to apply for several diseases such as uterine fibroid and prostatic hyperplasia. Furthermore, a combination of ultrasound and microbubbles is studied for a novel drug delivery system.

Microbubbles are used as an ultrasound contrast agent and composed of insoluble gas and covered shells such as lipids and protein^{15,16)} (Fig. 1A). In ultrasound imaging, ultrasound exposure induces an oscillation of microbubble, and ultrasound waves are deformed, including higher harmonic waves. The imaging device detects the reflection of higher harmonic ultrasound waves derived from microbubbles and describes the tissue structure. Microbubbles are useful to detect micro-blood flow and are applied to diagnose the tumor. Ultrasound exposure with adequate energy induces stable cavitation, which is a repetition of expansion and contraction of microbubbles (Fig. 1B). Furthermore, ultrasound exposure with high energy induces inertial cavitation, which is a collapse of microbubbles, resulting in the induction of phenomena such as microjet stream (Fig. 1B). The cavitation of microbubbles can enhance the permeability of the cellular membrane and blood vessel^{8,17-20)} (Fig. 1B). Although some parts of the mechanism

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Fig. 1. Enhancement of Vascular Permeability by Microbubbles and Ultrasound

(A) Microbubble is composed of insoluble gas coated with a shell, such as lipids and protein. We have developed microbubbles containing DSPG with high stability. (B) Ultrasound exposure induces constriction, expansion, or collapse of microbubbles. These mechanical forces affect endothelial cells and enhance vascular permeability.

are still unknown, microbubble behavior plays an important role in inducing transient pores on the cellular membrane, the temporal breakdown of tight junctions between endothelial cells, or increased endocytosis and transcytosis.^{19–23)} An enhancement of vascular permeability can improve delivery efficiency of low molecular weight compounds, genes, and proteins into the cells and tissues.^{24–26)} Our groups have also reported that the combination of ultrasound and microbubbles would be helpful in drug delivery and improvement of therapeutic effects.^{27–31)}

Stability of microbubbles is one of the important properties to accomplish increased vascular permeability followed by drug delivery because microbubbles have to circulate in blood flow in targeted tissue after systematic administration. An inner gas of microbubbles passes a shell and gradually dissolves in the water outside of the microbubble. Therefore, the shell composition influences the leakage of gas and stability of microbubbles. A head group of phospholipids affects lipid membrane stability, and membrane containing phosphatidylglycerol head group shows high stability.³²⁾ Therefore, focus was based on phosphatidyl glycerol as examination was conducted on the effect of phospholipid composition of shell on microbubbles stability.¹⁶⁾ We prepared microbubbles composed of 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine (DSPC), 1,2-distearoyl-*sn*-glycero-3-phosphatidylglycerol (DSPG) and 1,2-disetearoyl-*sn*-glycero-3-phosphoethanolamine-polyeth-

ylenglycol2000 (DSPE-PEG2000) containing perfluoropropane (Fig. 1A). When the shell composition of DSPC, DSPG, and DSPE-PEG2000 was in molar ratio of 30:60:10, microbubbles containing 60% DSPG showed high stability *in vitro* compared with other microbubbles which is prepared in different shell compositions. Additionally, to evaluate the circulation of microbubble containing 60% DSPG in blood flow, the echogenicity in the kidney was observed using ultrasound imaging device in a harmonic imaging mode after intravenous injection of microbubbles in mice. The microbubbles containing 60% DSPG showed higher echogenicity for a long time after injection than other microbubbles in different shell compositions. The results suggest that the microbubbles containing 60% DSPG have high stability *in vitro* and *in vivo*. The fabricated microbubbles would be suitable for ultrasound diagnosis and drug delivery. Thus, we have tried developing an efficient drug delivery system using microbubbles with ultrasound.

3. BLOOD–BRAIN BARRIER OPENING

In the past decades, the medical field has dramatically developed, and some diseases have been treatable. Nevertheless, diseases of the brain and central nervous systems such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and brain tumors still require advanced therapy. Several promising drugs have been studied using peptides, proteins, genes, nucleic acids, or cells. To treat diseases of the brain and central nervous system, it is important to develop not only high effective drugs but also delivery system because most drugs cannot reach the brain parenchyma after systemic administration. In the brain, blood–brain barrier restricts the transportation of molecules between the blood and brain parenchyma. The endothelial cells in the brain have a tight junction, and the interaction of pericytes and astrocytes resulted in no transportation of most drugs except low molecular weight and high hydrophobicity drugs^{33,34)} (Fig. 2A). Therefore, blood–brain barrier is a significant obstacle for drug delivery to the brain *via* systemic administration. To overcome this obstacle, drug delivery systems using ultrasound and microbubbles have been studied. The mechanical forces that especially expansion and contraction of microbubbles induced by ultrasound exposure can temporarily disrupt the tight junction between the endothelial cells and enhance the permeability of blood–brain barriers^{21,22)} (Fig. 2A).

We have also tried developing a blood–brain barrier-opening system using microbubbles and ultrasound.³⁵⁾ To evaluate blood–brain barrier opening, a mixture of Evans blue as a model drug and microbubbles was intravenously injected. Immediately, ultrasound was exposed to the right side of the brain. The blood was perfused after an hour, and then transferred Evans blue in each side of the brain was determined. As a result, the Evans blue amount in the right side of the brain, which was exposed to ultrasound, was higher than that of the left side. Furthermore, we examined the damage to the brain by the treatment of microbubbles and ultrasound. Hematoxylin–eosin staining revealed that the combination of microbubbles and ultrasound did not induce the extravasation of erythrocytes into the parenchyma. The results suggested that the combination of microbubbles and ultrasound could induce blood–brain barrier opening without notable damage. Ultrasound devices have been dramatically

Fig. 2. Blood-Brain Barrier Opening by Microbubbles and Ultrasound

(A) Mechanical forces of microbubbles induced by ultrasound exposure can temporally disrupt tight junctions between endothelial cells and enhance the permeability of blood-brain barrier. (B) A mixture of Evans blue (100 mg/kg) and microbubbles (3×10^9 particles/kg) was intravenously injected into the mouse. Immediately, ultrasound (Frequency: 3 MHz, Intensity: 0.5 W/cm^2 , Time: 3 min) was exposed to the right side of the brain. After one hour, blood was transcardially perfused, and the whole brain and slice brain were observed. (C) The delivered Evans blue into each side of the brain was extracted and measured. Data were shown as a box plot. $**p < 0.01$. Reprinted with modification from Omata *et al.* with permission.³⁰⁾ (Color figure can be accessed in the online version.)

improved in diagnosis and therapy. To induce blood-brain barrier opening, not only ultrasound devices but also microbubbles are essential. It is reported that the characteristics of microbubbles such as particle size and composition of the shell affect the blood-brain barrier opening.^{36,37)} Therefore, the development of optimized microbubbles is important to achieve efficient and noninvasive blood-brain barrier opening. Still, the effect of encapsulating gas in microbubbles on blood-brain barrier has not been evaluated. Thus, we prepared different gas-encapsulated microbubbles and examined the effect of encapsulated gas on blood-brain barrier opening.³⁰⁾ Three types of gas which are used in approved microbubbles were chosen. Perfluoropropane (C_3F_8), perfluorobutane (C_4F_{10}),

and sulfur hexafluoride (SF_6) are used in Definity, Sonazoid, and SonoVue, respectively. We fabricated C_3F_8 -encapsulated microbubbles (MB- C_3F_8), C_4F_{10} -encapsulated microbubbles (MB- C_4F_{10}), and SF_6 -encapsulated microbubbles (MB- SF_6). The average diameter of these microbubbles was $2\text{--}3 \mu\text{m}$, and the lipid composition of the shell was the same. Therefore, it was considered that the effect of gas could be compared. To evaluate the stability of microbubbles, ultrasound imaging was conducted and the echogenicity derived from microbubbles by contrast mode was supervised.

In *in vitro/vivo* studies, the echogenicity of MB- C_4F_{10} sustained was compared with MB- C_3F_8 and MB- SF_6 , and the echogenicity of microbubbles in the kidney of mouse was observed, while the echogenicity of MB- C_4F_{10} and MB- C_3F_8 was sustained compared with that of MB- SF_6 . These results suggested that MB- C_3F_8 and MB- C_4F_{10} were stable *in vitro* and *in vivo* compared with MB- SF_6 . The solubility of C_3F_8 and C_4F_{10} in water is lower than that of SF_6 . The prepared microbubbles had the same lipid composition in shell and similar size, and MB- C_3F_8 and MB- C_4F_{10} had less leakage of gas through the shell because of less solubility of C_3F_8 and C_4F_{10} in water. Furthermore, examination was performed on the different gas-encapsulated microbubbles on blood-brain barrier opening. The mixture of microbubbles and Evans blue was intravenously injected into the mouse, and immediately transcranial ultrasound exposure was performed to the right side of the brain. After one hour, the blood was transcardially perfused, and Evans blue delivered to the brain were determined. The amount of Evans blue delivered to the brain treated by MB- C_3F_8 or MB- C_4F_{10} with ultrasound were higher than that of MB- SF_6 (Figs. 2B, C). These results suggested that the MB- C_3F_8 and MB- C_4F_{10} with high stability *in vivo* would be suitable for blood-brain barrier opening. Furthermore, we evaluated the damage to the brain by hematoxylin-eosin staining and fluoro-jade C staining, which can detect denatured neurons. The experiments revealed that the MB- C_3F_8 and MB- C_4F_{10} with ultrasound did not induce notable damage to the brain. Our study demonstrated that C_3F_8 or C_4F_{10} encapsulated in microbubbles would be suitable to achieve efficient and noninvasive blood-brain barrier opening. Optimizing the characteristics of microbubbles, such as the composition of shells, particle size, and encapsulated gas helps establish a useful blood-brain barrier-opening system.

The blood-brain barrier opening with microbubbles and ultrasound has been applied to brain-related diseases such as cancer, Parkinson's disease, and Alzheimer's disease. The combination of microbubbles and ultrasound could deliver low molecular weight compounds and large molecules such as antibody.²⁵⁾ Nucleic acid with a large molecular weight is expected to treat intractable diseases and needs a delivery system to exhibit therapeutic effects because of physical characteristics such as negative charge and large molecular weight. The combination of microbubbles and ultrasound has the possibility to deliver a nucleic acid to the brain by enhancing the blood vessel's permeability. Kinoshita *et al.* examined nucleic acid delivery to the brain using microbubbles and ultrasound, followed by protein expression to reduce oxidative stress.²⁸⁾ Oxidative stress induces the degradation of neurons in some diseases. Glutathione shows an antioxidative activity and protects the degradation of neuron.^{38,39)} Glutathione synthesis needed by cysteine, and cysteine uptake are regulated

by excitatory amino acid carrier 1 (EAAC1).⁴⁰⁾ Therefore, the upregulation of EAAC1 increases cysteine, resulting in an increase of glutathione. EAAC1 expression is suppressed by miR-96-5p.⁴¹⁾ Thus, the antisense oligonucleotide inhibiting miR-96-5p would become neuroprotective drugs. Kinoshita *et al.* administrated the mixture of this antisense oligonucleotide and microbubbles into the internal carotid artery and simultaneously exposed ultrasound to the brain. Immunohistochemistry analysis revealed that EAAC1 expression treated by antisense oligonucleotide with microbubbles and ultrasound was increased compared with antisense oligonucleotide alone and antisense oligonucleotide as a negative control with microbubbles and ultrasound. Furthermore, intracellular glutathione treated by antisense oligonucleotide with microbubbles and ultrasound was higher than that of antisense oligonucleotide alone. The results suggested that the combination of microbubbles and ultrasound could efficiently deliver antisense oligonucleotides into the brain parenchyma, and the delivered antisense oligonucleotides could modulate the protein expression followed by physiological response. Thus, the combination of microbubbles and ultrasound would be useful to enhance the permeability of blood–brain barriers and deliver drugs such as antisense oligonucleotides into the brain parenchyma.

To establish blood–brain barrier opening system in clinic, many studies have progressed worldwide. Magnetic resonance-guided focused ultrasound combined with microbubbles has been clinically studied for glioblastoma therapy and others.^{12,14)} In this system, an ultrasound device is set on the head and transcranially applied without surgery because ultrasound frequency is low, and ultrasound can pass the skull. Alternatively, non-focused ultrasound has also been developed for glioblastoma therapy.¹³⁾ Here, the transducer is embedded under the skull with surgery. Therefore, it has some invasiveness, but it does not need to be set in each treatment and equipped with simple treatment. Additionally, a focused ultrasound device has been developed which can detect inertial cavitation and control the acoustic power not to inertial cavitation for safe treatment.⁴²⁾ Microbubbles have also been studied to achieve efficient and safe treatment. As previously described, the lipid composition of shell, encapsulated gas, and particle size of microbubbles could affect the blood–brain barrier opening.^{30,36,37)} Therefore, it is also important to develop the optimized microbubbles for blood–brain barrier opening to achieve a therapeutic system for brain-related diseases. Although some concerns should be addressed, a development of ultrasound device and microbubbles will help achieve efficient and safe blood–brain barrier systems followed by treatment of brain and central nervous system diseases.

4. BLOOD–TUMOR BARRIER OPENING

Chemotherapy is one of the therapeutic strategies for cancer therapy. The chemotherapy is including conventional chemical drugs and targeted molecular drugs. Although chemical conventional medicines such as doxorubicin (DOX) and cisplatin (CDDP) are widely applied for various types of cancer, they frequently introduce severe toxicities and side effects. To avoid their appearance, reduction of the injection dose would be effective. Although the dose reduction can decrease the frequency of side effects, it would be reduced therapeutic effect.

To get out of the dilemma, it is necessary to change the pharmacokinetics (PK) of the drug. There are some approaches for changing PK, such as modulation of the microenvironment in tumor tissue to enhance drug accumulation using the drug delivery carriers. Cancer cells in tumor tissue take essential nutrition and oxygen *via* blood flow. For the survival of cancer cells, tumor tissue needs angiogenesis. The tight junctions of neovascular tumor tissue are loose and result in leakages even for large molecules and nanomedicine. This is a different big property compared with vascular in normal tissue. In normal tissue, vascular permeability is limited for large molecules. This difference in vessel structure between tumor and normal tissue is used for drug delivery system.

In tumor tissue, large molecules and nanomedicine, which has a diameter of 100–200 nm, preferentially enhance extravasation and retention in tumor tissue. This phenomenon is known as the enhanced permeability and retention (EPR) effect.⁴³⁾ The EPR effect is an accumulation mechanism of nanomedicine such as liposomal doxorubicin (DOXIL). It was thought that the EPR effect was an attractive drug targeting strategy. DOXIL has been approved for Kaposi's sarcoma in which a practical EPR effect is observed in the clinic. However, reports have deduced that the mean delivery efficiency in nanomedicine based on EPR effect was only 0.7%.⁴⁴⁾ EPR effect is not sufficient to achieve the therapeutic effect with chemotherapy in many types of cancer. The limitation of the EPR effect is caused by the lack of fenestrations in the tumor endothelium, the rich extracellular matrix and stroma in tumor tissue, tumor interstitial fluid pressure, irregular vascularity, heterogeneous pericyte coverage, and poor blood flow within tumor, *etc.*^{45–47)} Especially, pancreatic cancers have a rich extracellular matrix and poor blood flow. In pancreatic cancer, drug delivery efficiency is commonly low and results in a low therapeutic effect even with gemcitabine or FOLFIRINOX (bolus injection and infusion of 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin), which is a first-line for pancreatic cancer or an optimal combination of chemotherapy.⁴⁸⁾ Altogether, it is thought that the EPR effect is rare in clinic, anti-tumor drugs, and nanomedicine would be difficult to be delivered into tumor tissue across the blood vessels in most types of tumors. It means that the blood–tumor barrier would exist and endothelial cells have functioned as blood–tumor barrier. To get over blood–tumor barrier and achieve the effective drug delivery into tumor tissue, it is crucial to develop a new approach to promote extravasation by enhancing the neovascular permeability in tumor tissue.

As mentioned in the previous section, the combination of microbubbles and ultrasound can effectively deliver small and large molecules into the brain by opening the blood–brain barrier. It is expected that this approach could be applied to anti-tumor drug delivery by blood–tumor barrier opening. Ueno *et al.* reported improvement in anti-tumor effect with chemotherapy for osteosarcoma.⁴⁹⁾ Osteosarcoma is the most frequent malignant tumor. DOX is considered one of the most effective drugs. In the chemotherapeutic regimens, a high dosage of the drug requires eliminating tumor successfully. Adversely, chemotherapy under high dose would frequently cause the side effects such as cardiotoxicity, immune suppression, nephrotoxicity, and others. To prevent the side effect, alternative strategies for treating osteosarcoma should be developed. Under this situation, it was developed

Fig. 3. Blood-Tumor Barrier Opening by Microbubbles and Ultrasound

Effects of bubble ultrasound-mediated drug delivery system (BUS-DDS) on *in vivo* anti-tumor activity of drugs (cisplatin (CDDP) and pegylated liposomal doxorubicin (PLD)) applied to HeLa and HEC1B cells on day zero (white arrow). * $p < 0.05$. (A) BUS-DDS (CDDP), HEC1B. Growth of HEC1B cell xenografts upon treatment with 0.05 and 0.5 mg/kg CDDP under BUS-DDS (B) BUS-DDS (CDDP), HeLa. The growth of HeLa cells xenografts upon treatment with 0.05 and 0.5 mg/kg CDDP under BUS-DDS (C) BUS-DDS (PLD). The growth of HeLa cell xenografts upon treatment with 0.2 and 2.0 mg/kg of PLD under BUS-DDS. (D) Treatment protocol indicating that anti-cancer drugs were injected with 40 μ L lipid-stabilized bubbles (LB) from the tail vein. The ultrasound probe was placed on the tumor, and ultrasound exposure was simultaneously initiated. LB (40 μ L) was repeatedly injected every 3 min (total dose: 400 μ L). The duration of ultrasound exposure was 30 min. (Color figure can be accessed in the online version.)

an effective therapeutic system for osteosarcoma by DOX delivery with submicron bubbles (Bubble liposome) and ultrasound. Interestingly, this system achieved an equivalent tumor growth suppression at about 1/5 the dose of DOX employed in monotherapy. Additionally, a significant reduction of platelet number was observed in the monotherapy with DOX but not in our delivery system at the injection dose with an equivalent therapeutic effect. Moreover, Yamaguchi *et al.* attempted to deliver CDDP for cervical cancer (HeLa) (Fig. 3A) and uterine endometrial cancer (HEC1B) (Fig. 3B) in mouse xenograft model with lipid-stabilized microbubbles and diagnostic ultrasound scanner.²⁷⁾ This bubble and ultrasound-mediated drug delivery system (BUS-DDS) significantly enhanced anti-tumor effects without any adverse events. Furthermore, Hirabayashi *et al.* developed anti-epidermal growth factor receptor (EGFR) antibody-conjugated microbubbles (EGFR-MB). In the human gingival squamous carcinoma xenograft model, bleomycin delivery by combining ultrasound and EGFR-MB exhibited remarkable anti-tumor activity.⁵⁰⁾ These findings suggest the possibility of proposing a novel strategy for chemotherapy.

Kotopoulos and colleagues have conducted a pilot study, which is first-in-human to deliver the anti-tumor drug with microbubbles and ultrasound.^{51,52)} In the study, gemcitabine delivery was performed for inoperable pancreatic cancer patients to evaluate safety and potential toxicity. In this Phase I trial, gemcitabine was intravenously infused over 30 min. After that, ultrasound was exposed with a commercial clinical

ultrasound scanner (1.9 MHz, mechanical index (MI): 0.2, duty cycle 1%) for 31.5 min under ultrasound guidance to target the location of pancreatic cancer. And SonoVue, which has been an approved microbubble as a contrast agent by European Medicines Agency, was utilized. This combination significantly improved median survival from 8.9 to 17.6 months with no additional toxicity than gemcitabine alone. This pilot study has proved the concept of a new drug delivery system by combining microbubbles and ultrasound in humans. Recently, Kotopoulos *et al.* reported acoustic cluster therapy (ACT) as a novel drug delivery system by the combination of a new formulation of microbubbles and ultrasound for pancreatic ductal adenocarcinoma (PDAC).⁵³⁾ The ACT is an ultrasound-activated formulation combining negatively charged, commercially available, and clinically employed microbubbles (Sonazoid, GE Healthcare, U.K.) with positively charged microdroplets.

The microdroplets consist of a fully or partially fluorinated or halogenated low blood soluble oil that has a boiling temperature of $< 50^{\circ}\text{C}$. A mixture of these microbubbles and microdroplets makes a small cluster of microbubble-microdroplets by the electrostatic force. The clusters are intended for co-injection with a drug. A lipophilic drug may optionally be embedded in the microdroplets. When the clusters are exposed with ultrasound in tumor tissue, the volumetrically oscillating microbubbles (Sonazoid) transfer energy to the microdroplet, initiating vaporization. As a result, 20–30 μ m bubbles have been formed in capillary vessels, induced ultrasound-guided,

and targeted drug release. In this event, the permeability of the capillary vessel would be enhanced and resulted in effective delivery of the drug into tumor tissue. In the PDAC xenograft subcutaneous model, the combination of ACT and paclitaxel injection and ultrasound exposure could effectively suppress tumor growth. This research group also reported colon cancer therapy by ACT along with irinotecan.⁵⁴⁾ In the Royal Marsden Hospital in the UK, Phase I trial on the ACT with chemotherapy for treatment of liver metastasis in patients with solid tumors with an expansion cohort in metastatic colorectal and pancreatic cancer has been started.⁵⁵⁾

During the early stages of research with microbubbles and ultrasound, small molecule delivery has been mainly reported. In recent research, there are many reports about the delivery of middle and large molecules such as small interfering RNA (siRNA),⁵⁶⁾ microRNA (miRNA),³¹⁾ plasmid DNA,⁵⁷⁾ antibodies and nanoparticles.^{29,58–60)} Generally, siRNA and miRNA are unstable in the bloodstream due to degradation by ribonuclease (RNase). To prevent degradation, their molecules are loaded with microbubbles or nanoparticles. When ultrasound is exposed to their molecules-loaded microbubbles or nanoparticles combined with microbubbles in the bloodstream of tumor tissue, the loaded molecules is quickly released at the sonication site. Simultaneously, sonoporation would open the tight junction of capillary vessels and result in the effective delivery of siRNA, miRNA, or their molecules-loaded nanoparticles into tumor tissue. This delivery system could be applied to nanomedicine such as DOXIL. Our collaborators reported anti-tumor effects in various types of tumors in mice^{27,60)} (Fig. 3C) or dog.²⁹⁾ In these experiments, anti-tumor effects were observed. From these results, it was suggested that the combination of microbubbles and ultrasound could open pores of sizes which will be at least 100 nm enough to pass through the nanomedicine on neovascular in tumor tissue.

The drug delivery technologies for cancer therapy with microbubbles and ultrasound are low invasive. They could apply for various types of drugs, from small and large molecules to nanomedicine as nanoparticles. The therapeutic effects could enhance and reduce the adverse effects because of help achieve “reinforced targeting.” Therefore, it is expected that this technology would be an attractive drug delivery system for various therapeutic agents and nanomedicine for cancer therapy.

5. CONCLUSION

Microbubbles and ultrasound could enhance the permeability of blood–brain barrier and tumor neovascular, and improve drug delivery to the targeted tissues. In the review, we described only the brain and tumor, while many studies have been conducted against other tissues such as the liver, kidney, and other kind type of tumor with promising results. Therefore, microbubbles and ultrasound would be useful drug delivery systems by enhancing blood vascular permeability. One of the advantages is that the system could be applied for many types of tissues and drugs because ultrasound is externally for the targeted tissue from the body. The mechanism of drug delivery is enhancing vascular permeability by mechanical forces derived from microbubbles. Microbubbles and ultrasound are also used for diagnosis in the clinic and seemed to be a promising system applied for theranostics that achieves

therapy and diagnostics. Additionally, microbubbles and ultrasound can induce many biological effects, which ablate the tumor tissues and enhance neuron activity.^{61,62)} Although advanced studies are still required, microbubbles and ultrasound can apply for many purposes.

In conclusion, the combination of microbubbles and ultrasound could enhance vascular permeability, resulting in improved drug delivery to the targeted tissues. The mechanism of enhancing permeability is that the expansion, contraction, and collapse of microbubbles are induced by ultrasound exposure. Many fundamental studies have been conducted, and some clinical studies have also progressed. Although there are still some issues in establishing a drug delivery system in clinics, it is believed that these issues would be solved, and a novel therapeutic approach would be installed.

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