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REVIEW ARTICLE

Ultrasonic drug delivery in Oncology

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Summary

Ultrasound-assisted drug delivery is an emerging technique that has the advantage of being non-invasive, efficiently and specifically targeted and controllable. While systemic drugs often show detrimental side effects, their ultrasound-triggered local release at the selected tissue may improve safety and specifity of therapy. An increasing amount of animal and preclinical studies demonstrates how ultrasound can also be used for increasing the efficacy of chemotherapeutic drug release to solid tumors. In particular, this technique may be functional to reach uniform delivery of chemotherapeutic agents throughout tumors, which is naturally restricted by their abnormal vascularization and interstitial pressure.

This review deals with the physical mechanisms of ultrasound, the different kinds of drug carriers (microbubbles, liposomes and micelles) and the biological phenomena useful for cancer treatment (hyperthermia, sonoporation, enhanced extravasation, sonophoresis and blood-brain barrier disruption), showing how much ultrasonic drug delivery is a promising method in the oncological field.

Key words: *drug delivery, cancer, microbubbles, ultrasound*

Mechanisms of action

Physics of ultrasound

Ultrasound consists of mechanical pressure waves which propagate through various media. The difference between ordinary audio sound and ultrasound is the frequency range, being in the latter above the audible threshold of 20,000 cycles per second, or Hertz (Hz). The intensity of ultrasound wave is measured in Watts/cm^2. Like other foms of wave energy, ultrasound can be focused, reflected, refracted and absorbed. Differently from electromagnetic radiations, ultrasonic waves are actual movement of molecules which are compressed (at high pressure) and expanded (at low pressure). As ultrasound is absorbed during propagation through a medium, it deposits energy in the form of heat. However, ultrasonic waves are absorbed relatively little by water and biological tissues, in comparison to electromagnetic waves.

Medical use of ultrasound can be divided into diagnostic, surgical and therapeutic. In diagnostics, very low intensities (1-50 mW/cm^2) are used to avoid tissue heating, with frequencies ranging between 3 and 5 MHz. Surgical ultrasound instead, is characterised by very low frequencies (20-60 kHz) and very high intensities (above 8 W/cm^2). Finally, therapeutic ultrasound is used mostly in physiotherapy at frequencies around 0.7 to 3 MHz at intensities of 0.5 to 3 W/cm^2 [1].

Hyperthermia

The intensity of ultrasound wave represents the energy of mechanical vibrations of the medium particles, or more exactly the power carried per cross section area of the beam. If the beam is focused on a small portion of the target tissue, the power per area becomes very high and significant

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Table 1. Characteristics of commercially available microbubbles

Name	*Manufacturer*	*Shell material*	*Gas*	*Mean size (μm)*
Albunex®	Molecular Biosystems	Albumin	Air	4.3
BiSphere™	Point Biomedical	Albumin	Air	2.0–6.0
Definity®	Bristol-Myers Squibb	Phospholipids	C_3F_8	1.1–3.3
EchoGen®	Sonus Pharmaceuticals	Albumin	C_5F_{12}	2.0–5.0
Imagent®	Imcor	Phospholipids	N_2/ C_6F_{14}	6.0
Imagify™	Acusphere	PLGA/phospholipids	C_4F_{10}	2.2
Levovist®	Schering	Galactolipids	Air	2.0–4.0
MicroMarker™	VisualSonics	Phospholipids	C_4F_{10}	2.0–3.0
Optison™	GE Healthcare	Albumin	C_3F_8	2.0–4.5
Sonazoid™	GE Healthcare	Phospholipids	C_4F_{10}	2.4–3.6
SonoVue®	Bracco	Phospholipids	SF_6	2.0–3.0
Targestar™	Targeson	Phospholipids	C_4F_{10}	2.5

PLGA:poly(lactic-co-glycolic) acid, C_3F_8:Octafluoropropane, C_4F_{10}:Perfluorobutane, C_5F_{12}:Perfluoropentane, C_6F_{14}: Perfluorohexane, SF_6:Sulfur hexafluoride

thermal energy can be absorbed, causing heating of the sonicated tissue. This is the case of High-Intensity Focused Ultrasound (HIFU), a therapeutic procedure approved in many countries, used to ablate tumors such as uterine fibroids and prostate cancer. This type of ultrasound provokes in the focal region pressures of up to 70 MPa and an intensity of 100-10,000 W/cm^2, which raise the temperature within the tissue above 65 °C and causes coagulation necrosis [2]. HIFU may also be used to create high temperatures not necessarily to treat the cancer alone, but in conjunction with targeted delivery of cancer drugs (see *liposomes* further down).

Cavitation

Cavitation is a physical phenomenon consisting in the formation of vapor zones within a fluid. This happens because the local pressure drops until it reaches the vapor pressure of the liquid itself, which undergoes a phase change, forming a microbubble (cavity) containing vapor. Microbubbles may also be introduced in the form of ultrasound contrast agents such as Albunex (Molecular Biosystems, San Diego, CA), BiSphere (Point Biomedical, San Carlos, CA), Definity (Bristol-Myers Squibb Medical Imaging, Billerica, MA), EchoGen (Sonus Pharmaceuticals, Bothell, WA), Imagent (Imcor Pharmaceuticals, San Diego, CA), Imagify (Acusphere, Watertown, MA), Levovist (Schering, Berlin, Germany), Micromarker (Visualsonics, Toronto, Canada), Optison (GE Healthcare, Princeton, NJ), Sonazoid (GE Healthcare) and SonoVue (Bracco, Princeton, NJ). Their shell usually are made of albumin (Albunex, BiSphere, EchoGen and Optison), galactolipids (Levovist) or phospholipids (Definity, Imagent, Micromarker, Sonazoid and Sonovue) and they contain an inert gas. Table 1 shows the characteristics of commercial ultrasound contrast agents.

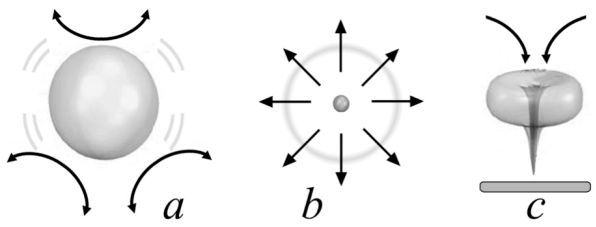

Figure 1. Different types of cavitation: **a)** Stable cavitation-arrows indicate microstreaming; **b)** Inertial cavitation - arrows indicate the shock waves; **c)** asymmetric cavitation near a rigid surface.

Microbubbles are forced to oscillate in the presence of an acoustic field, expanding at low pressure and contracting at high pressure. If the intensity of the acoustic field is insufficient to cause total bubble collapse, this type of cavitation is called " non-inertial " or " stable ". These oscillations create a circulating fluid flow (called microstreaming) around the bubble (Figure 1a) with velocities and shear rates proportional to the amplitude of the oscillations [3].

If the acoustic intensity increases, the amplitude of oscillation may increase to a point in which the inward moving wall of fluid has sufficient inertia that it cannot reverse direction when the acoustic pressure reverses, but continues to

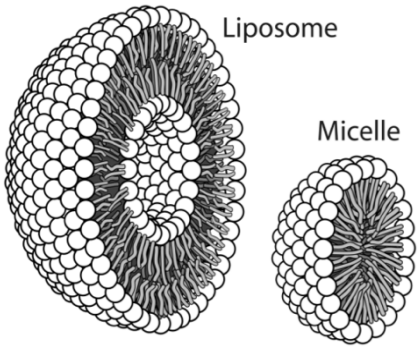

Figure 2. Liposome and micelle; hydrophilic heads in white, hydrophobic tails in grey (Image credit: Mariana Ruiz Villarreal).

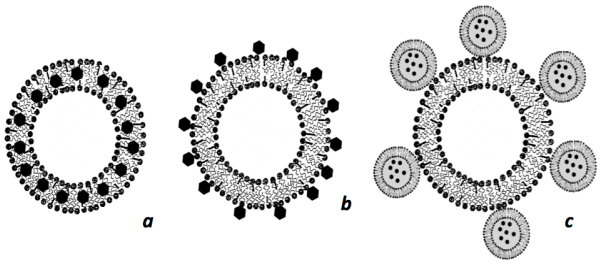

Figure 3. Drug-carrying microbubbles: **a)** drug molecules are inside the hydrophobic shell; **b)** drug molecules are attached to the shell through electrostatic binding; **c)** drug-containing liposome are covalently attached to the microbubble's surface.

compress the gas in the bubble. As the bubble shrinks, the pressure and temperature of the vapor within increases. The bubble eventually collapses to a minute fraction of its original size, at which point the gas within dissipates into the surrounding liquid via a rather violent mechanism, which releases a significant amount of energy in the form of an acoustic shock wave and as visible light (Figure 1b). Just before total collapse, the temperature of the vapor within the bubble may reach several thousand Kelvin, and the pressure several hundred atmospheres. The collapsed bubble often fragments into smaller bubbles that serve as cavitation nuclei, grow in size, and eventually collapse again. This form of cavitation is called " inertial " or " transient ". If the bubble collapses near a rigid boundary (such as a blood wall vessel), the bubble is pierced from one side by the formation of a supersonic microjet leading towards the boundary (Figure 1c). This kind of bubble collapse is called " asymmetric cavitation" [4].

Sonochemistry

Cavitation is a violent phenomenon that concentrates the energy from ultrasound into a small volume. This process can generate shear forces and microstreaming that are able to give sonolysis effects and free radical production proportional to energy dose transferred by ultrasonic waves, which may lead to cytotoxic effects and the onset of apoptosis [5].

Drug carriers

In order to avoid the interaction between non-tumor tissues and the delivered drug, the latter can be attached to a carrier from which it is released at the target site. These drug carriers comprise microbubbles, liposomes and micelles. All of them have an average size less than that of red blood cells, thus they are capable of penetrating even into the small blood capillaries. Microbubbles differ from other carriers as they are filled with gases (usually air or perfluoropropane). Liposomes are similar to microbubbles, but their interior is liquid instead of gaseous. They are composed of a lipid bilayer, so the interior of the liposome is hydrophilic and the intramembrane region is hydrophobic. Micelles instead are made of a single layer of lipids, therefore their interior is hydrophobic (Figure 2).

Microbubbles

Using drug delivery from microbubbles by ultrasound gives not only the possibility to avoid uptake in unsonicated tissue, thus reducing side-effects, but also to visualize the drug-loaded microbubbles using low-pressure ultrasounds (remember that microbubbles were born as ultrasound contrast agents). This promising technique is called "image guided drug delivery" [6]. Moreover, sonication can be used to induce at the same time both local drug release and cell membrane permeabilization (see *Sonoporation* further down).

Microbubbles can be loaded either incorporating drug molecules inside the hydrophobic shell (Figure 3a) or attaching them to the shell through electrostatic binding (Figure 3b). Another way is to attach drug-containing liposome to the microbubble's surface (Figure 3c).

In an *in vivo* rat model, sonication of doxorubicin-shell-embedded microbubbles led to a 12-fold increase in local drug concentration and significant reduction in tumor growth [7]. Likewise, tumor-bearing mice administered microbubbles

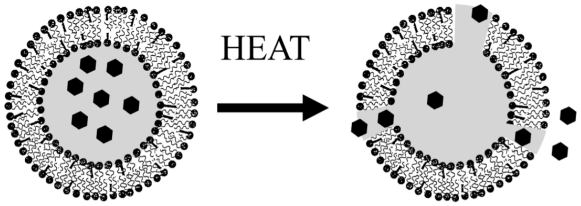

Figure 4. Thermosensitive liposome.

loaded with 10-hydroxycamptothecin inside the shell and exposed to ultrasound, showed a remarkable drug accumulation in tumor tissues and a significant increase in tumor inhibition rate [8]. Microbubbles loaded with carmustine decreased tumor progression and improved survival of glioma-bearing rats [9]. In a recent research, Ren et al. [10] developed a novel docetaxel-loaded microbubble which proved to be both an efficient ultrasound contrast agent *in vivo* and enhanced the antitumor effect of this drug *in vitro*. As well as incorporating drugs within the shell, another technique involves the inclusion of a drug-containing oil-phase within the microbubble [11].

The useful method of attaching drug-loaded liposomes to the microbubbles' surface (Figure 3c) through avidin-biotin binding allows to create a higher drug loading capacity [12]. In fact, microbubbles loaded with doxorubicin-liposomes killed, after exposure to ultrasound, twice more melanoma cells compared to doxorubicin-liposomes alone [13]. However, although avidin-biotin binding is an easy way to obtain liposome-loaded microbubbles, it limits the *in vivo* application due to the immunogenic nature of the avidin molecule. This can be overcome assembling liposomes covalently bound to the microbubbles [14].

In addition to small molecules like the aforementioned chemotherapeutic drugs, also therapeutic nucleotides (genes and siRNA) can be loaded onto/into microbubbles. In the presence of cavitation-inducing ultrasound (1 MHz, 10% duty cycle, 2W/cm^2), microbubbles with siRNA conjugated to their surface silenced more luciferase expression (90%) than the free siRNA alone (10%) in HUH7 cells [15]. Similarly, siRNA-loaded microbubbles in the presence of ultrasound (1 MHz, 20% duty cycle, 2W/cm^2) were able to knockdown twice more the tumor suppressor gene *PTEN* than control siRNA alone [16]. Endo-Takahashi et al. [17] developed polyethyleneglycol (PEG)-modified bubble liposomes that contain ultrasound-contrast gas and entrapping inside them pDNA or siRNA. In this way, *in vivo* applicability was improved, protecting the nucleic acids from degradation exerted by nuclease present in the serum. Moreover, these microbubbles can be simultaneously used for ultrasound imaging and gene delivery, thus useful in the field of theranostics (a combination of therapeutics and diagnostics). Carson et al. [18] developed microbubbles containing perfluorobutane with herpes simplex thymidine kinase (HSVtk) plasmid attached to the surface and showed that such attachment protected from degradation. HSVtk is widely used as a suicide gene as it gives cancer cells sensitivity to ganciclovir (GCV) by encoding a protein that metabolises non-toxic GCV into a phosphorylated product. This produces a toxic nucleoside analogue that induces apoptosis by inhibiting the function of DNA polymerases [19]. Mice bearing C3H/NeJ carcinoma were injected with these HSVtk-plasmid-loaded microbubbles and a 1.3 MHz ultrasound at 1.8MPa was applied to the tumor region. An increased expression of the payload gene and a delay in tumor growth was detected by the authors [18].

One limitation to the use of microbubbles is due to their dimensions: being generally above 1 μm (Table 1), it is likely that they do not extravasate into tumors, as the interstices between tumor-associated endothelial cells are in the range of 500 nm [20]. In order to avoid this drawback, "nanobubbles" small enough to extravasate through these endothelial gaps were created. Kang and Yeh [21] developed such nanobubbles loaded with carmustine and observed a decrease in size of rat brain tumor as a result of ultrasound-aided drug delivery.

Liposomes

Because liposomes do not contain gases they are not echogenic and cannot undergo cavitation. On the other hand, they are of interest in oncology because of their small size, being usually between 100 and 400 nm [22]. Thermally [23] and mechanically [22, 24-28] triggered release of drugs loaded in liposomes have been reported (Figure 4). Weinstein was the first to develop thermosensitive liposomes for treating solid tumors in mice [29]. More recently, it was demonstrated that therapeutic ultrasound can serve as a source of hyperthermia and trigger doxorubicin release from liposomes [30]. These thermosensitive lipos-

omes release entrapped drugs when they reach a temperature of 42 °C [31]. Ning et al. showed that ultrasound-induced hyperthermia, besides increasing drug anti-tumor activity, accelerated the release of doxorubicin from long-circulating liposomes [32]. By increasing the temperature from 37 °C to 41 °C, the rate of release of doxorubicin was increased 6-fold after one hour of sonication at 2 W/cm^2. The accumulation of doxorubicin in RIF-1 tumor cells was 10 times higher when introduced in liposomes at 42 °C compared to when introduced as free drug at 37° C. Several others reports have shown that other drugs can be released from liposomes using ultrasonic hyperthermia [33-37]. The way to modify phospholipid composition in order to allow mechanical disruption by sonication has been recently indicated [24-25]. Nevertheless, the exposure parameters of the ultrasound used in these studies (40 kHz, 100% duty cycle, up to 6 min) are unlikely to be applicable in therapy; at the end of the day, mechanically triggered release requires the inclusion of gas in order to establish cavitational phenomena.

Micelles

The minimal diameter of a liposome is around 80 nm and is determined by the maximal tolerated proximity of the phospholipid headgroups imposed by the curvature of the inner layer [38]. Micelles instead have a typical size of 10-50 nm, do not contain gases (as liposomes do not) and (as explained above) can contain only hydrophobic drugs. Their small size and consequent ability to extravasate at the tumor site has been used to improve site-specific drug delivery in cancer therapy [39-42].

A combination of 70 kHz ultrasound and micelle-encapsulated doxorubicin have been shown to increase the cytotoxicity of the drug *in vitro* [43,44]. Moreover, sonication can enhance the intracellular uptake of micelles and its internalization into HL60 cells [45]. Concerning *in vivo* studies, Nelson et al. [46] showed that exposure to 70-kHz ultrasound of doxorubicin encapsulated in Plurogel™ micelles significantly decreased the size of colorectal cancer tumors in rats. When unencapsulated doxorubicin was administered, the same dose was lethal to the rats within two weeks from injection. Finally, two studies using ovarian cancer-bearing nu-nu mice as tumor models showed that micelle accumulation was significantly higher in the sonicated tumors than in the non-sonicated [47,48].

Sonoporation

Ultrasound can be used to temporarily permeabilize the cell membrane allowing for the uptake of drugs, DNA and other therapeutic compounds from the extracellular environment [49]. This membrane alteration is transient, leaving the compound trapped inside the cell after sonication. Sonoporation, unlike other methods of transfection or chemotherapy, combines the capability of improving drug and gene transfer with the possibility of restricting this effect to the desired area and the desired time. Thus, sonoporation is a promising drug delivery and gene therapy technique. Although the biophysical mechanism that results in the cell membrane permeability change needs further elucidation, it seems that sonoporation is not due to inertial cavitation, but to micro-streaming and shear stresses related to stable oscillations [50].

Several *in vitro* studies have shown that ultrasound-induced membrane permeability increases the uptake of anti-cancer drugs such as bleomycin and adriamycin [51-53]. Watanabe et al. [54] demonstrated that microbubbles in the presence of low-intensity 1-MHz pulsed ultrasound enhanced the delivery of cisplatin and its cytotoxic effect on tumor cells, both *in vitro* and *in vivo*. In a similar study, Sorace et al. [55] found that *in vitro* maximal uptake of extracellular molecules occurred at a transmission frequency of 1.0 MHz; then, combining *in vivo* the use of taxol with microbubbles sonicated with these parameters, the authors found that cancer cell death increased by 50% over chemotherapy alone. Finally, it should be added that although *in vitro* there is sufficiently dissolved gas and enough organic molecules with a surfactant nature that the sonication itself generates cavitation bubbles which may induce sonoporation, this would be hardly possible *in vivo*, since the lungs are very efficient at clearing out small bubbles from the circulatory system [56]. Therefore, to induce sonoporation *in vivo*, microbubbles are indispensable.

Besides use with chemotherapeutic small molecules, sonoporation may be particularly useful for the delivery of free nucleotides which would otherwise be prevented to cross the plasma membrane due to their large size and net negative charge. Some works showed that ultrasound-mediated transfection can be an effective gene delivery tool into tumor *in vivo*, being an attractive approach in cancer gene therapy as the method is minimally-invasive and tumor specific gene transfer, requiring only exposure to ultrasound

applied to the surface of the body [57,58].

Manome et al. injected a naked plasmid with a reporter gene into MC38 colon carcinoma in mice [59]. Application of 1 MHz ultrasound increased the reporter activity 3-fold over the non-insonated control. Higher power densities increased reporter activity, as did increasing insonation time up to 30 sec. Anwer et al. [60] delivered an IL-12 gene to a mouse tumor model, reporting that sonication significantly increased the gene expression, with transfected tissue being limited to the tumor vasculature. The expression of IL-12 was sufficient to inhibit tumor growth compared with the control conditions. Delivering a naked plasmid DNA reporter gene into subcutaneous Dunning prostate tumors in rats using 0.85 MHz ultrasounds, Huber et al. [57] detected a 15-fold increase in reporter activity compared to non-insonated controls.

In the end, it should be underlined that there is no clear consensus on the duration of the existence of ultrasound-created membrane pores. While some authors detected that this phenomenon lasts in the order of seconds to minutes [61,62], Yudina et al. [63] asserted that pore opening lasts up to 24 hours. Based on this finding, the latter authors suggested that it could be more advantageous first to sonoporate the tissue, followed by delivery of the drug.

Other mechanisms

Enhanced extravasation

The vasculature around tumor sites is inherently leaky due to the rapid vascularization necessary to serve fast-growing tumors. These characteristics lead to abnormal molecular and fluid transport dynamics known as "enhanced permeability and retention (EPR) effect" [64]: certain sizes of molecules (typically liposomes, nanoparticles, and macromolecular drugs) tend to accumulate in tumor tissue much more than they do in normal tissues.

Taniyama et al. [65] reported that sonication with 1 MHz, 0.4 W for 30 sec with Optison enhanced the transfection efficiency of naked plasmid DNA into skeletal muscle cells *in vivo* as well as *in vitro*. Using a chorioallantoic membrane model *in vivo*, Stieger et al. demonstrated that convection is the dominant transport mechanism enhancing vascular permeability and delivery of therapeutic agents [66]. Using a MC38 tumor model, Bohmer et al. [67] evaluated the effects of focused ultrasound on vessel permeability as a function of pressure, number of cycles and type of microbubble. Ultrasound-mediated microbubble destruction enhanced particularly the extravasation in the highly vascularized outer part of the MC38 tumor and adjacent muscle and would, therefore, be most useful for release of, for instance, anti-angiogenic drugs. Many other studies have shown that ultrasound in the presence of microbubbles enhances the extravasation of drugs into solid tumors [68-70].

The first to show the mechanism by which ultrasound can enhance the extravasation of drugs was Arvanitis et al. [71], who identified inertial cavitation as the main phenomenon. Further studies were performed by Bazan-Peregrino et al. [72], who developed a 3D tumor-mimicking model consisting in a vessel running through agar gel containing breast cancer cells. Once introduced the oncolytic adenovirus AdEHE2F-Luc through the phantom vessel, acoustic cavitation was initiated by a 0.5 MHz HIFU transducer, in the presence and absence of the contrast agent SonoVue. Ultrasonic pressures were chosen to maximize either broadband emissions, associated with inertial cavitation (1.25 MPa, 6.5% duty cycle and PRF 10 Hz), or ultraharmonic emissions, associated with stable cavitation (360 kPa, 90% duty cycle and PRF of 10 Hz), while varying duty cycle to keep the total acoustic energy delivered constant for comparison across exposures. In the absence of ultrasound, few cancer cells were infected by the oncolytic virus. Conversely, the induction of cavitation determined an increased extravasation, resulting in increased adenovirus infection efficacy. Stable cavitation doubled the number of viral particles extravasated, which determined a 10-fold increase in transgene expression at 24 hrs. Moreover, inertial cavitation determined a 4-fold increase in viral concentration which caused a 200-fold increase in luciferase expression.

Sonophoresis

Therapeutic ultrasounds (1-3 MHz, 1-3 W/cm^2) have been used for years to deliver low molecular weight drugs as well as macromolecules into or through the skin [73]. Mitragotri et al. [74] showed that low frequency ultrasound was much more effective than higher frequencies and provided evidence as to the mechanism involved: skin permeability increased with decreasing frequency, and with increasing time of exposure and intensity, indicating cavitation as the underlying mechanism [75].

So far, sonophoresis has been used for a wide variety of drugs, but only one study used this

technique with an anti-cancer substance [76]. Moreover, in this study sonication produced a decrease in percutaneous drug penetration, because of a diffusive loss of the hydrophilic drug (5-fluorouracil) from the skin surface into the overlying volume of coupling gel.

Blood-brain barrier disruption

The ability to deliver therapeutic agents to the brain is limited by the blood-brain barrier (BBB), a specific structure of the blood vessel wall that hinders transport and diffusion from the vasculature to the brain. The BBB is formed by the brain capillary endothelium and is impassable for 98% of all small-molecule drugs and virtually 100% of large-molecule agents. Only small-molecule drugs with a mass under a 400-500 Da and highly lipophilic can cross the BBB in therapeutically significant amounts [77]. Transcranial delivery of low-frequency ultrasound can be used to temporarily disrupt the BBB and thus enhance drug diffusion [78]. Usually, ultrasonic exposure burst at 10 msec with pressure amplitudes less than 1 MPa are used for durations of 20-30 sec repeated at the frequency of 1 Hz [79]. Administration of microbubbles further lowers the frequency threshold for BBB disruptions, thus allowing for much lower and safer frequencies to be used [80].

Targeted BBB disruption could also aid in the delivery of chemotherapeutic agents in brain tumors. The first study investigated the delivery of liposome-encapsulated doxorubicin which normally does not penetrate the BBB and detected increased concentrations in the sonicated locations of rat brains (burst length=10 msec, PRF=1 Hz, sonication duration=120 sec and frequency=1.5 or 1.7 MHz) [81]. These regions showed significantly higher concentrations of doxorubicin than the contralateral side. The concentration of the drug in the brain tissue was observed to increase linearly with increasing microbubble concentration. Moreover, focused ultrasound in combination with magnetic nanoparticles has been shown to allow delivery of epirubicin across the BBB in a mouse model [82]. Similarly, focused ultrasound significantly enhanced the penetration of carmustine through the BBB in normal and tumor-implanted rat brains, resulting in control of tumor progression and enhanced survival of glioblastoma-bearing animals [83].

Perspectives

In the last decade, research in ultrasound-activated drug delivery saw a significant growth as a result of the introduction of gas bubbles. In addition to this, owing to the fact that microbubbles can be simultaneously used as drug-vehicles and contrast agents, they are beginning to be used as theranostic tools (theranostics is an emerging field that combines drug therapy and diagnosis such as ultrasound imaging). Among the many therapeutic applications, ultrasound-assisted drug delivery is proposed also for use in oncology. This treatment allows targeted release, improved delivery and enhanced extravasation into solid tumours.

Beside being a non-invasive therapy which improves the efficiency and specificity of oncological treatment, several issues need to be solved. Details of how ultrasound permeabilizes the cell membrane and the temporal window of this effect are needed. Moreover, the ability of ultrasound to cause a stress response (similar to heat shock) that may enhance or interfere with the action of drugs should be thoroughly investigated.

So far, microbubbles and liposomes are used in drug delivery as they are thermodynamically stable, but they are cleared out by the reticulo-endothelial system. On the other hand, micelles are not cleared by the reticulo-endothelial system, but they are thermodynamically unstable when diluted in blood. Thus, an ongoing challenge is the improvement of stealth and stability of the delivery vehicles. The development of sonosensitive solid nanoparticles is also a promising alternative which is meeting increasing interest. In general, the production of delivery vehicles below 500 nm is essential to allow optimal extravasation into the tumor interstitial space.

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