Asian Journal of Pharmacy and Life Science ISSN 2231 – 4423
Vol. 1 (3), July-Sept, 2011

ULTRASOUND MEDIATED DRUG DELIVERY SYSTEM

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ABSTRACT

Ultrasound has received less attention than other imaging modalities for molecular imaging, but has a number of potential advantages. It is cheap, widely available and portable. Using Doppler methods, flow information can be obtained easily and non-invasively. It is arguably the most physiological modality, able to image structure and function with less sedation than other modalities. This means that function is minimally disturbed, and multiple repeat studies or the effect of interventions can easily be assessed. High frame rates of over 200 frames a second are achievable on current commercial systems, allowing for convenient cardiac studies in small animals. It can be used to guide interventional or invasive studies, such as needle placement. Ultrasound is also unique in being both an imaging and therapeutic tool and its value in gene therapy has received much recent interest. Ultrasound biomicroscopy has been used for in utero imaging and can guide injection of virus and cells. Ultra high frequency ultrasound can be used to determine cell mechanical properties. The development of micro bubble contrast agents has opened many new opportunities, including new functional imaging methods, the ability to image capillary flow and the possibility of molecular targeting using labeled micro bubbles.

Key words: Ultrasound mediated drug delivery system, Dignostic application, Ultrasonography, 3D ultrasound, Drug delivery carriers.

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Received: 07/09/2011 Accepted:20/09/2011

INTRODUCTION

Ultrasound is the most widely used cross-sectional imaging modality worldwide. Important recent developments include micro bubble contrast agents, quantitative approaches and new signal processing and display methods. In the field of Ultrasound mediated drug delivery system, the used drug must satisfy two requirements in order to be successful. First, the drug must be effective in the environment it is used for. And second, it must reach the target site in optimal quantities. In the field of drug delivery, it has great importance to have sufficient drug accumulation at the diseased site. To obtain this goal it is important that at first, a high concentration of the administered dose at the diseased site has to be present and second, the drug has to be able to extravagate homogeneously at the region to be treated. Ultrasound can be used in different ways to improve these problems. To be able to develop new or better strategies for delivering drugs with the use of ultrasound it is extremely important to understand the underlying mechanisms. There are two interrelated aspects in this research. First, in order to be able to control the process there has to understand about the physical interactions induced with ultrasound. And second, it

is important to determine which bio effects occur as a response on these physical mechanisms. Ultrasound contrast agents (UCAs) can be used to improve imaging by introducing a material with different acoustic properties from that of tissues. The most common approach is the use of intravenous injections of small air or gas bubbles (micro bubbles) that boost the Doppler signal from blood vessels. The original impetus for their development (to enhance Doppler signals) has been largely superseded by a number of niche "micro bubble-specific" applications[1]. Micro bubbles can be quantified in passive and active imaging approaches. In passive approaches, low acoustic power is used to observe the enhancement effects without disrupting micro bubbles. The intensity changes produced by them can be quantified and in some situations are proportional to relative micro bubble concentration [2].The active approach uses a transient pulse of high acoustic power to destroy micro bubbles and then observe refilling into an area of interest during an infusion. One method is to repeatedly pulse ultrasound at varying triggering intervals, waiting for a steady state, and study the relationship between steady-state enhancement levels and triggering delay settings [3-4].

Ultrasound is the transmission of pressure waves through a medium, just like normal sound only with a frequency greater than the upper limit of human hearing, approximately 20 kilohertz and up. These waves can be reflected, refracted, focused and absorbed. Ultrasound is able to actually move molecules; the material gets compressed or expanded at the changing pressure of the wave. The absorption of ultrasound energy in the body is relatively low compared to other forms of electromagnetic radiation. Therefore it can penetrate deep inside the body, which is necessary for medical treatments. Also x-rays and radiofrequency pulses can penetrate enough into the body; the interaction of ultrasound with tissue comes to expression in different forms, heat generation cavitations and acoustic radiation force. These mechanisms cause different bio-effects on the tissue which can be used to enhance the drug uptake and concentration at target site. [5]

Advantages

Several advantages are accompanied with the use of ultrasound as a drug delivering method. First of all, ultrasound is a non-invasive technique with an external source. Second, it can be applied locally at a very small region of interest inside of the body. Third, it is able to reach deep inside of the human body, in absence of harming the tissue in the beam path. And it does not necessarily require the development of new drugs.

Drug delivery

The first challenge is to overcome dose limiting factors that are caused by the systemic toxicity of the used drug. This is mostly due to the non-specific nature and spread of the drug throughout the blood circulation of the body. This causes the drug to also accumulate in healthy tissue. The dose in the target tissue has not the desired concentration and it is not possible to increase the overall dose. This limits the effectiveness of the drug in the diseased tissue and treatment is far from optimal. It is well known that local administration of drugs is a promising strategy, so several solutions have been proposed to increase the target concentration. These methods can be divided into three groups; ‘active targeting drug delivery’, ‘passive targeting drug delivery’ and ‘physical targeting drug delivery’.

Active targeting is usually achieved by combining the drug particle with a targeting moiety, like antigen–antibody and ligand–receptor binding this result in preferred accumulation of the drug in the targeted region. Passive targeting takes advantage of the differences in permeability between tissues, allowing the drug to accumulate at regions with higher permeability. Passive targeting also includes the administration of drugs exactly at the desired place, for example invasively into an organ artery.

Physical targeting makes use of an external trigger, such as ultrasound or magnetic fields to release the drug at a desired region. In the past most research was performed on the active targeting of drugs to the target region, but in de past few decades increasing numbers of studies are dedicated to passive and physical targeting of drugs, because of the huge improvement of concentrating drug in a very small region. Ultrasound mediated local drug delivery utilizes a form of passive targeting and/or physical targeting for drug delivery. The second challenge is to make sure the drug can enter the diseased tissue efficiently, in other words, specific barriers that inhibit the drug to pass, have to be opened or lifted. In most cases the particles are too large to cross barriers, such as vascular tissue and the blood-brain-barrier.

To overcome these barriers a modification of the target environment is required. The interaction of ultrasound with tissue causes increased permeability in several ways. The vascular wall can be ruptured and as a result drug particles can pass through. Next to that the vascular wall can have increased permeability without being ruptured. A big problem with drug molecules and particles is their inability to distribute homogeneously in a adequate concentration in the diseased cells. This is due to the fact that it has to pass several barriers, before reaching the diseased cells. The transport of the drug will here be explained for tumours, as they are widely investigated and play a big role in drug delivery. When a drug is administered intra venous, Ultrasound will enter blood circulation of the tumour after a certain period. Then it first distributes through the vascular space of the tumour.

Medical ultrasonography

Diagnostic sonography (ultrasonography) is an ultrasound-based diagnostic imaging technique used for visualizing subcutaneous body structures including tendons, muscles, joints, vessels and internal organs for possible pathology or lesions. Obstetric sonography is commonly used during pregnancy and is widely recognized by the public.

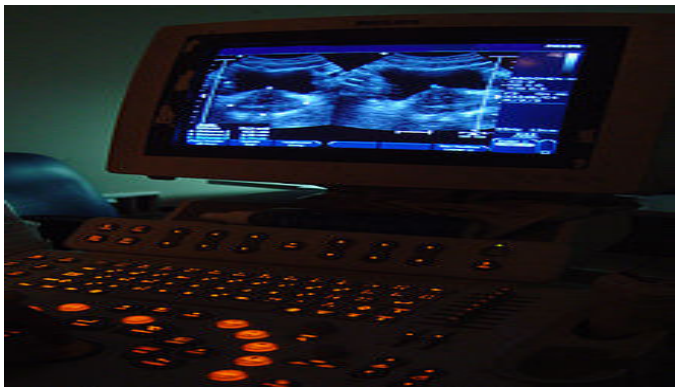

Fig.1 Medical ultrasonography technique

Diagnostic applications

Typical diagnostic sonographic scanners operate in the frequency range of 2 to 18 megahertz, though frequencies up to 50-100 megahertz has been used experimentally in a technique known as biomicroscopy in special regions, such as the

anterior chamber of eye. The choice of frequency is a trade-off between spatial resolution of the image and imaging depth: lower frequencies produce less resolution but image deeper into the body. Higher frequency sound waves have a smaller wavelength and thus are capable of reflecting or scattering from smaller structures. Higher frequency sound waves also have a larger attenuation coefficient and thus are more readily absorbed in tissue, limiting the depth of penetration of the sound wave into the body [6].

Therapeutic applications

Therapeutic applications use ultrasound to bring heat or agitation into the body. Therefore much higher energies are used than in diagnostic ultrasound. In many cases the range of frequencies used are also very different.

- Ultrasound is sometimes used to clean teeth in dental hygiene.
- Ultrasound sources may be used to generate regional heating and mechanical changes in biological tissue, e.g. in occupational therapy, physical therapy and cancer treatment. However the use of ultrasound in the treatment of musculoskeletal conditions has fallen out of favor.
- Focused ultrasound may be used to generate highly localized heating to treat cysts and tumors (benign or malignant), This is known as Focused Ultrasound Surgery (FUS) or High Intensity Focused Ultrasound (HIFU). These procedures generally use lower frequencies than medical diagnostic ultrasound (from 250 kHz to 2000 kHz), but significantly higher energies. HIFU treatment is often guided by MRI.
- Focused ultrasound may be used to break up kidney stones by lithotripsy.
- Ultrasound may be used for cataract treatment by phacoemulsification.
- Additional physiological effects of low-intensity ultrasound have recently been discovered, e.g. its ability to stimulate bone-growth and its potential to disrupt the blood-brain barrier for drug delivery.
- Procoagulant at 5-12 MHz,

Ultrasonic biomicroscopy (40–200 MHz)

Turnbull demonstrated the first use of UBM for non-invasive in uterus imaging of live mouse embryos and further showed the method to be effective in detecting and quantifying the midhindbrain deletion associated with a null mutation of Wnt-1.More recent investigations in this area have focused on high frequency ultrasound imaging and Doppler methods for examine mouse cardiovascular development.

A number of genes have been identified in the mouse that is critical for normal development of the cardiovascular system. Duplex Doppler permits detailed examination of flow patterns in the developing heart.. Umbilical blood vessels can be identified from in uterus UBM images, as they form the connection between the embryo and the placenta. In addition, the moving blood in the vessels is highly echogenic, resulting in a prominent appearance of the moving speckle pattern on real-time UBM images. [7] Duplex UBM has also the potential to investigate the growth and differentiation of tumours in vivo. Tumor volume can be measured as a function of time and therapeutic intervention can be accurately monitored in the mouse.[8]

Modes of ultrasound

Several different modes of ultrasound are used in medical imaging. These include:

- A-mode: A-mode is the simplest type of ultrasound. A single transducer scans a line through the body with the echoes plotted on screen as a function of depth. Therapeutic ultrasound aimed at a specific tumor or calculus is also A-mode, to allow for pinpoint accurate focus of the destructive wave energy.
- B-mode: In B-mode ultrasound, a linear array of transducers simultaneously scans a plane through the body that can be viewed as a two-dimensional image on screen.
- C-mode: A C-mode image is formed in a plane normal to a B-mode image. A gate that selects data from a specific depth from an A-mode line is used; then the transducer is moved in the 2D plane to sample the entire region at this fixed depth. When the transducer traverses the area in a spiral, an area of 100 cm^2 can be scanned in around 10 seconds.
- M-mode: M stands for motion. Ultrasound pulses are emitted in quick succession - each time, either an A-mode or B-mode image is taken. Over time, this is analogous to recording a video in ultrasound. As the organ boundaries that produce reflections move relative to the probe, this can be used to determine the velocity of specific organ structures.
- Doppler mode: This mode makes use of the Doppler effect in measuring and visualizing blood flow
    - Color Doppler: Velocity information is presented as a color coded overlay on top of a B-mode image
    - Continuous Doppler: Doppler information is sampled along a line through the body, and all velocities detected at each time point is presented (on a time line)
    - Pulsed wave (PW) Doppler: Doppler information is sampled from only a small sample volume (defined in 2D image), and presented on a timeline[9]

Ultrasound-Mediated DNA Transformation System

There have been relatively few reported successes in the transformation of thermophilic, Gram-positive, anaerobes (TGPAs). Firstly, some thermophilic bacteria are difficult to transform due to the unique features of their cell envelope, the formation of endospore and the low permeability of plasma membrane. Secondly, most of the reported gene transfer protocols for these organisms were based on electroporation, where higher electroporation efficiency was limited to only a few laboratories that used sophisticated customer-built cuvettes and pulse generator. Key drawbacks of electroporation include the need for an ion-free condition and the requirement of contact between electrodes and solution. Moreover, DNA electroporation through the membrane is not a mechanical penetration process, because interactions between pore and DNA are involved. This interaction may slow down DNA penetration through cell wall after pulsing, which might expose the foreign double-stranded DNA to high-temperature and reduce its stability and integrity. Thirdly, the fastidious requirements in cell handling and preparation of competent-cells for

strictly anaerobic bacteria further exasperated this situation. Therefore, there is a tremendous need for developing a simple, rapid and effective method for genetically transforming TGPAs. [10]

Ultrasound-triggered image-guided therapy

The local delivery of therapeutic molecules mediated by ultrasound is a novel approach to addressing unmet clinical needs by providing a minimally-invasive platform for targeted delivery of pharmaceuticals. It enables high concentrations of drugs to be deposited under image guidance to specific locations in the body. It can, therefore, be a preferred treatment option for non-systemic diseases. This produces more constant and controlled drug concentration profiles with favorable pharmacokinetics. With the advancement in imaging techniques, local drug delivery can now be applied in the human body with an unprecedented spatial and temporal resolution. Apart from delivering drugs to the desired location, another advantage of using ultrasound is the increase of cellular uptake through an effect called sonoporation, which gives the method an additional advantage over needle- or catheter-based procedures. Ultrasound for therapy is preferably highly focused, in order to expose a well-defined area, while steering of the ultrasound beam should be flexible and fast to allow for the controlled exposure of a region of interest with a potentially complex shape. Two types of ultrasound-mediated delivery systems can be distinguished: pressure-mediated delivery and temperature-mediated delivery. Pressure-mediated delivery is performed using micro bubbles, currently clinically applied as ultrasound contrast agents that react to short ultrasound pulses by oscillation or bubble destruction. As this is a fast process and the micro bubbles and their destruction can be conveniently imaged using ultrasound, ultrasound imaging is the preferred modality for guiding the drug delivery. In the case of temperature-mediated delivery, in which ultrasound is applied for a sufficient amount of time to establish a local temperature increase, magnetic resonance imaging (MRI) is the most appropriate modality for image guidance, as it offers the possibility of therapy planning as well as monitoring of the local temperature distribution during the drug delivery procedure. The first application of high intensity focused ultrasound (HIFU) in combination with MRI was in the ablation of uterine fibroids, which gives an excellent starting point for temperature monitoring during HIFU treatment. For temperature-induced delivery we present material systems, where drug and imaging agent are both encapsulated in temperature-sensitive carriers that allow the monitoring of temperature-sensitive delivery vehicles using MRI and disintegrate upon temperature increase. For pressure-sensitive micro bubble agents we discuss the options of drug delivery with either co-injected drugs or drugs incorporated into the micro bubble carrier, and focus on the induced permeability increase of cells. Finally an outlook on potential applications is given, ranging from the improvement in small-molecule drug delivery to gene-silencing using small interference RNA as a new therapeutic drug format. [11]

Temperature-triggered, MRI guided drug delivery

During ultrasound energy deposition, the actual tissue temperature is difficult to predict. Physiological processes may modify local heat conduction and energy absorption. Blood flow may increase during temperature increase and thus

change heat conduction. Unlike ablation treatments, the exposure to an elevated temperature for drug delivery using ultrasound should remain well below the damage threshold. In order to improve the therapeutic efficiency and the safety of the intervention, real-time mapping of temperature and thermal dose with MRI appear to offer the best strategy to optimize such intervention [12]

Transdermal ultrasound mediated drug delivery

Several methods exist for improving transdermal drug delivery such as chemical mediation using liposome or chemical enhancers, and physical mechanisms such as iontophoresis, lasers, electroporation, micro needles and ultrasound (also called sonophoresis or phonophoresis). Numerous studies of these methods have shown that ultrasound mediated transdermal drug delivery offers promising potential for noninvasive drug administration. However the broad literature on ultrasound drug delivery is not confined only to transdermal applications. A large body of work focuses specifically on delivery to internal organs, but does not cover tissues or gene delivery. Additionally for transdermal work, the reader is also directed to several well written arti#les for further reading and additional viewpoints on this topic. Passive drug delivery across the stratum corneum can be transported with molecules that have a weight less than 500 Da. In general the stratum corneum, which varies in thickness (≈ 10–20 μm) depending on the body location, forms the barrier to drug diffusion. This low permeability is attributed to the outermost skin layer that consists of a compact and organized structure of cells named keratinocites surrounded by lipid bilayers. Ultrasound enhanced transdermal drug delivery offers advantages over traditional injection drug delivery methods which are invasive and painful. Currently few drugs, proteins or peptides have been successfully administered transdermally for clinical applications because of the low skin permeability to these relatively large molecules. However from a research viewpoint, the list of compounds which have been shown to transdermally cross skin via ultrasound is ever increasing. One hypothesis indicates that once the drug has traversed the stratum corneum, the next layer is easier to cross and subsequently the drug can reach the capillary vessels to be absorbed.

Liposome as a drug delivery system

Liposome are composite structures made of phospholipids and may contain small amounts of other molecules. Though liposome can vary in size from low micrometer range to tens of micrometers, unilamellar liposome, as pictured here, are typically in the lower size range with various targeting legends' attached to their surface allowing for their surface-attachment and accumulation in pathological areas for treatment of disease. Liposomes are artificially prepared vesicles made of lipid bilayer. Liposome's can be filled with drugs, and used to deliver drugs for cancer and other diseases. Liposome's can be prepared by disrupting biological membranes, for example by sonication. [13]

Liposome can be composed of naturally derived phospholipids with mixed lipid chains (like egg phosphatidyl ethanolamine) or other surfactants. [14]

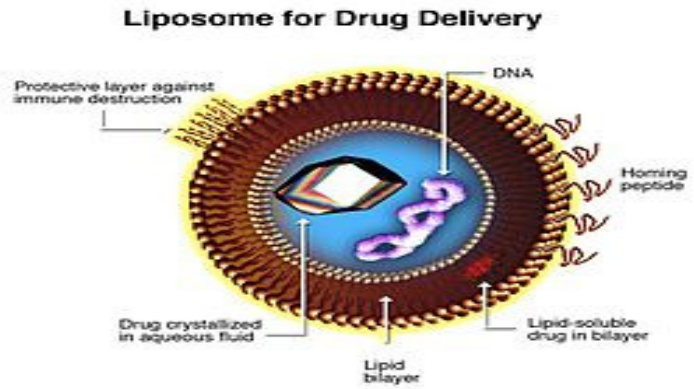

Fig. 2 Liposome for ultrasound drug delivery

High-intensity focused ultrasound

"HIFU (High-Intensity Focused Ultrasound or High Frequency Ultrasound) (sometimes FUS or HIFUS) or high frequency ultrasound is a highly precise medical procedure using high-intensity focused ultrasound to heat and destroy pathogenic tissue rapidly. It is one modality of therapeutic ultrasound, and, although it induces hyperthermia, it should not be confused with this technique, which heats much less rapidly and to much lower therapeutic temperatures (in general < 45°C)."

Clinical HIFU procedures are typically image-guided to permit treatment planning and targeting before applying a therapeutic or ablative level of ultrasound energy. When MRI is used for guidance, the technique is sometimes called Magnetic Resonance-guided Focused Ultrasound, often shortened to MRgFU. When ultrasonography is used, the technique is sometimes called Ultrasound-guided Focused Ultrasound, often shortened to USgFUS. Magnetic resonance imaging (MRI) is used to identify tumors or fibroids in the body, before they are destroyed by the ultrasound. [14]

Therapeutic ultrasound is a minimally invasive or non-invasive method to deposit acoustic energy into tissue. Applications include tissue ablation (HIFU) (for tumor treatments, for example), hyperthermia treatments (low-level heating combined with radiation or chemotherapy), or the activation or enhanced delivery of drugs.

Advantages

High Intensity Focused Ultrasound is often considered a promising technology within the non-invasive or minimally invasive therapy segments of medical technology. HIFU's capacity to generate in-depth precise tissue necrosis using an external applicator, with no effect on the surrounding structures, is unique. The history of using therapeutic ultrasound dates back to early in the 20th century. Technology has continually improved and additional clinical applications, both diagnostic and therapeutic, have become an integral part of medicine today.

An important difference between HIFU and many other forms of focused energy, such as radiation therapy or radio surgery, is that the passage of ultrasound energy through intervening tissue has no apparent cumulative effect on that tissue.

3D ultrasound

There are several different scanning modes in medical and obstetric ultrasound. The standard common obstetric diagnostic mode is 2D scanning. "In 3D fetal scanning, however, instead of the sound waves being sent straight down and reflected back, they are sent at different angles." The returning echoes are processed by a sophisticated computer program resulting in a reconstructed three

dimensional volume image of fetus's surface or internal organs; in much the same way as a CT scan machine constructs a CT scan image from multiple x-rays. 3D ultrasounds allow one to see width, height and depth of images in much the same way as 3D movies but no movement is shown.

3D ultrasound is a medical ultrasound technique, often used in obstetric ultrasonography (during pregnancy), providing three dimensional images of the fetus.

3D ultrasound was first developed by Olaf von Ramm and Stephen Smith at Duke University in 1987. Clinical use of this technology is an area of intense research activity especially in fetal anomaly scanning but there are also popular uses that have been shown to improve fetal-maternal bonding 4D fetal ultrasounds are similar to 3D scans, with the difference associated with time: 4D allows a 3-dimensional picture in real time, rather than delayed, due to the lag associated with the computer constructed image, as in classic 3-dimensional ultrasound.

If the system is used only in the Obstetrics Application, the ultrasound energy is limited by the manufacturer below FDA limits for obstetrical ultrasound, whether scanning 2, 3 or 4 dimensionally. (The FDA limit for obstetrical ultrasound is 94 mW/cm^2 while there has been no conclusive evidence for harmful effects of 3D ultrasounds on a developing fetus, there still remains controversy over its use in non-medical situations, and generally, the AIUM recommends that 3D ultrasounds should be undertaken with the understanding that a risk may exist.

Micro bubbles as ultrasound contrast agents

Ultrasonography is a widely used diagnostic medical imaging technique that is non-invasive, relatively low-cost, easy to use, provides real-time imaging, and importantly, avoids the use of hazardous ionizing radiation. Ultrasound wave pulses generated by an ultrasound transducer are partially reflected or scattered by the interfaces between different tissues. The transducer detects the ultrasound waves returned by scattering, and these signals are converted to ultrasound images. Since blood scatters ultrasound poorly, ultrasound contrast agents, which increase the scattering and reflection of ultrasound waves, are utilized for blood flow imaging, especially in cardiosonography. Gramiak and Shah in 1968 were the first to use contrast agents in echocardiography[15] and reported that the aortic delineation was improved by intracardiac injection of agitated saline containing air bubbles. However, these air microspheres disappeared within a few seconds following intravenous injection due to the high solubility of air in blood, and the impossibility of larger air bubbles to pass through pulmonary capillaries. For these reasons, it is difficult to use injected conventional air bubbles for opacifying the left cardiac chambers, unless they are injected by the intracoronary or aortic route.

To improve the stability and decrease the size of air bubbles, microbubbles with a thin shell such as albumin (Albunex) or galactose palmitic acid (Levovist) have been developed. These bubbles are first-generation micro bubbles, and are air-filled microspheres. Their mean diameter ranges from 1 to 8 μm, and they are capable of passing through pulmonary capillaries. However, these air-filled micro bubbles disappear from the bloodstream within seconds after administration because of their

low resistance to arterial pressure gradients, and the high solubility of air in blood[16]. Approaches for increasing the stability of microbubbles and decreasing the solubility of their gas in blood are clearly required, and lead to the development of microbubbles filled with a high molecular weight hydrophobic gas such as perfluorocarbons or sulfur hexafluoride. These microbubbles represent second-generation contrast agents, in which surfactants, sonicated albumin and phospholipids are used to form the bubble shell in order to improve microbubble stability in the bloodstream. The acoustic backscatter of these microbubbles is higher than that of blood and organs, due to differences in acoustic impedance between gases, and blood or organs. Therefore, microbubbles are useful contrast agents, especially in echocardiography. In addition, Sonazoid which was a phosphatidylserine-stabilized perfluorobutane microbubbles was developed as a useful contrast agent for hepatic tumors [17], and. This is due to uniqueness of Sonazoid whose microbubbles are likely to be taken up by Kupffer cells (liver macrophages) in the healthy liver and enhances contrast of the liver parenchyma during the delayed phase, which usually occurs within 10 min after the injection. In contrast, tumor that lacks Kupffer cells was not enhanced resulting in clear negative contrast of the tumor. Thus, Sonazoid is a new type of microbubble which is able to target Kupffer cells.[18]

Increase the efficiency of cancer treatment

Laser-induced bubble formation around nanoparticles may play a crucial role in selective laser nanophotothermolysis of cancer cells targeted with nanoparticles. In this paper, we propose theoretically, and confirm experimentally, a new dynamic mode for selective cancer treatment that involves the overlapping of bubbles inside the cell volume. This bubbles-overlapping mode (BOM) can dramatically increase the efficiency of cancer treatment by laser-heated nanoparticles as a result of the large damage range. On the basis of nanoparticle optics below the diffraction limit and the kinetic model of bubble dynamics, we found the criteria and conditions (antiparticle distance and particle size and concentration) for BOM initiation in cancer cells by laser radiation. Using MDA-MB-231 breast cancer cells, we showed that the optimal size range of the gold nanoparticles for effective laser initiation of BOM is 30–40 nm and the lower concentration limit is n 2.44 × 1011 cm–3 (i.e. the absolute number of particles homogeneously distributed inside a tumor cell is n 430). It was demonstrated that the formation of nanoclusters on the cell surface with sizes larger than the sizes of individual nanoparticles, may further increase the efficiency of the laser treatment of cancer. [19]

Prostate cancer detection:

The diagnosis of prostate cancer is currently limited by the low sensitivity and specificity of systematic conventional grey-scale ultrasonography. We assessed contrast enhanced color Doppler ultrasonography by means of a micro bubble ultrasound contrast agent to detect tumor vascularity and improve the diagnosis of prostate cancer. The use of a Micro bubble ultrasound contrast agent for transrectal color Doppler targeted biopsy significantly improved the detection of prostate cancer compared with systematic biopsy following conventional grey-scale ultrasonography ($p<0.001$). Contrast-agent enhanced color Doppler imaging may allow for limited targeted biopsies (five or less), which reduces costs and morbidity.

Leukemia Treatment:

Acute myeloid leukemia (AML) is a quick progressive cancer which is characterized by neoplastic proliferation of myeloid cells. Current diagnostics of AML with prognostic ally and therapeutically implications includes cellular morphology, immunological markers, and in particular gene mutation analysis, cytogenetic, and response after chemotherapy. These high intensive therapy regimens involve toxicity problems for a lot of patients over 60 years and only 20-30% of the patients achieve long time survival. The need for new, effective and targeted treatment is therefore great. Imaging modalities play an important role in evaluating disease extension and progression in hematological malignancies. It also has a vital role in pre-clinical trials using animal models. Gas-filled micro bubbles covered with a bioactive substance pass harmlessly and uneventfully through blood vessels until they are exposed to ultrasound. Then, the bubbles burst, causing not only the release of the bioactive substance but also the opening of holes in the cells (sonoporation) that line the vessel.

Gene delivery:

Progress in cardiovascular gene therapy has been hampered by concerns over the safety and practicality of viral vectors and the inefficiency of current nonviral transfection techniques. Ultrasound exposure (USE) enhances transgene expression in vascular cells by up to 10-fold after naked DNA transfection, and enhances lipofection by up to three-fold. We report here that performing USE in the presence of micro bubble echo contrast agents enhances acoustic cavitations and is associated with approximately 300-fold increments in transgene expression after naked DNA transfections. This approach also enhances by four-fold the efficiency of polyplex transfection, yielding transgene expression levels

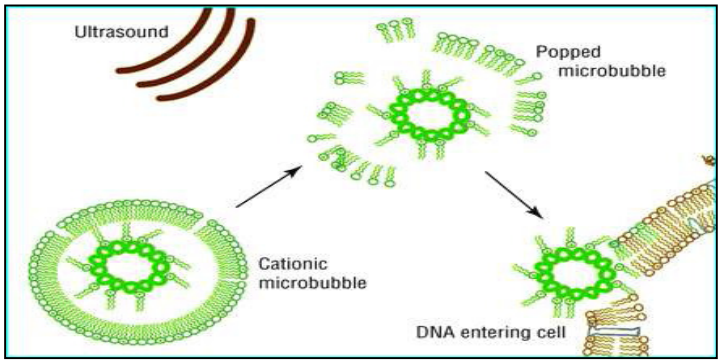

Fig.3 Gene delivery using ultrasound and micro bubbles

Approximately 3000-fold higher than after naked DNA alone. These data indicate an important role for acoustic cavitations in the effects of USE. Ultrasound can be focused upon almost any organ and hence this approach holds promise as a means to deliver targeted gene therapy in cardiovascular conditions such as such angioplasty rest enosis and in many other clinical situations. The presence of gas in the gene-filled micro bubble allows ultrasound energy to "pop" the bubble. An energetic wave is then created which allows the genetic material to enter Surrounding cells. Micro bubbles are currently used clinically as contrast agents in ultrasound (US) diagnostics and experimentally as drug or nucleic acid carriers [20].

CONCLUSION

Ultrasound has long been utilized as a useful diagnostic tool. Therapeutic ultrasound was recently developed and is being utilized in clinical settings. The combination of therapeutic ultrasound and nano/micro bubbles is an interesting and important system for establishing a novel and non-invasive

gene delivery system. Gene expression efficiency with this system can effectively deliver gene compared with conventional non-viral vector system such as lipofection method due to deliver gene into cytosol without endocytosis pathway. Many in vivo studies has been reported about ultrasound-mediated gene delivery with nano/micro bubbles. Especially, there are some reports about feasibility studies of gene therapy for various diseases and In addition, this system has a potency of site specific gene delivery by the control of ultrasound exposure site. Therefore, it is expected that this technology would be utilized as a novel gene delivery system in clinical field

The first application of ultrasound in the ablation of uterine fibroids, which gives an excellent starting point for temperature monitoring during HIFU treatment. Further clinical trials are running for delivery of doxorubicin using temperature-sensitive liposomes. New options to improve image-guided drug delivery will need new imaging technology, material development and an expansion of the knowledge on mechanisms of uptake of drugs into cells, especially for high molecular weight therapeutics. These complex questions are currently addressed in public-private consortia such as the European Framework 7 project “Sonodrugs”. Specific attention needs to be paid to the choice of drug and its formulation. Small-molecule drugs, such as doxorubicin and paclitaxel, are well established therapies. By depositing a larger dose at the region of interest, the efficacy of cancer therapy can be improved. Novel therapeutic formats, using DNA-based gene therapy or gene silencing by therapeutic siRNAs, need specific mechanisms to enter the cell, currently often by the use of viral vector. With sonoporation, a different way of entering the cell is introduced, avoiding potential adverse reactions and complex delivery systems such as viruses.

Although ultrasound-based drug delivery has only seen limited clinical use for transdermal drug delivery, there has been considerable momentum in research aimed at using ultrasound for a wide variety of medical applications. Ultrasound-mediated gene therapy using sonoporation and targeted delivery has progressed from in vitro proof-of-concept studies to produce biological effects in angiogenesis and diabetes studies. These techniques have also been used for cancer therapy, thrombolytic, and disruption of the blood-brain barrier in animal models. It is also worth noting that ultrasound can facilitate targeted drug delivery through thermal means, in addition to the mechanisms associated with cavitation. Ultrasound hyperthermia can be used to target thermally sensitive drug-carrying liposomes or to induce gene expression through localized heat shock response.

ACKNOWLEDGEMENT

Authors are grateful to the College of Pharmacy, IPS Academy, Indore, for providing the necessary facilities for successfully completion of the present work.

Table 1. List of clinically approved liposomal drugs

Name	Trade name	Company	Indication
Liposomal amphotericin B	Abelcet	Enzon	Fungal infections
Liposomal amphotericin B	Ambisome	Gilead Sciences	Fungal and protozoal infections
Liposomal cytarabine	Depocyt	Pacira (formerly SkyePharma)	Malignant lymphomatous meningitis
Liposomal daunorubicin	DaunoXome	Gilead Sciences	HIV-related Kaposi's sarcoma
Liposomal doxorubicin	Myocet	Zeneus	Combination therapy with cyclo phosphamide in metastatic breast cancer
Liposomal IRIV vaccine	Epaxal	Berna Biotech	Hepatitis A
Liposomal IRIV vaccine	Inflexal V	Berna Biotech	Influenza
Liposomal morphine	DepoDur	SkyePharma, Endo	Postsurgical analgesia

Table 2. Ultrasound contrast agents.

Name	Shell	Entrapping gas	Size
Albunex	Albumin	Air	4.3
Levovist	Galactose	Air	2-4
Optison	Albumin	Perfluoropropane	3-32
Definity	Lipids	Perfluoropropane	1.1-20
Imagent	Lipids	Perfluoropropane	5
Sonovue	Lipids	Sulphur hexafluoride	2.5
Sonazoid	Lipids	Perfluoropropane	2-3

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