by ultrasonic irradiation. Thus, there is significantly less risk in causing any systemic disorder. In addition, these drug act exclusively upon tumor tissues when combined with ultrasonic irradiation, with no adverse effect upon normal tissues. An important factor involved in ultrasound irradiation is the chemical reactions induced during the course of violent microbubble collapse. Short lived free radicals can be created by ultrasound that could alter various compounds leading to cell killing (Yumita, 2003). Sonoluminescence (the production of light by cavitation) may also be related to the complex sonochemical or sonodynamic reactions. However, the exact mechanism related to cytotoxicity still remains to be solved. Acoustic cavitation can chemically activate photosensitive drugs specifically bound to malignant cell membrane, which could result in cell surface disruption (Uchida *et al.*, 1997). Recent experiments with Adult T cell leukemia cells were specifically killed by low intensity ultrasound of 0.3 W/cm^2 in the presence of porfimer sodium (Tachibana *et al.*, 1997). Abe *et al.* (2002) developed a strategy for the selective destruction of cancer cells by ultrasonic irradiation in the presence of an antibody-conjugated photosensitizer. A photoimmunoconjugate (PIC) was prepared between ATX-70, a photosensitizer of a gallium-porphyrin analogue, and F11-39, a high affinity monoclonal antibody (MAb) against carcinoembryonic antigen (CEA), which is often overexpressed in various carcinoma cells. The conjugate, designated F39/ATX-70, retained immunoreactivity against purified CEA and CEA-expressing cells as determined by enzyme-linked immunosorbent assay, flow cytometry and immunofluorescence microscopic analysis. The cytotoxicity of F39/ATX-70 against CEA-expressing human gastric carcinoma cells *in vitro* was found to be greater than that of ATX-70, when applied in combination with ultrasound irradiation. *In vivo* anti tumor effects in a mouse xenograft model resulted in a marked growth inhibition of tumor, compared with ultrasound alone or ultrasound after administration of ATX-70. Arakawa *et al.* (2003) demonstrated that PAD-S31, a water-soluble, chlorin-derivative sonochemical sensitizer, can be used for sonodynamic therapy on neointimal hyperplasia in a rabbit stent model. One hour after the intravenous administration of PAD-S31, ultrasound energy (1 MHz, 0.3 W/cm^2) was delivered transdermally to the sonodynamic therapy group. At 28 days, all stent sites were analyzed morphometrically. The ratio of the intimal and medial cross-sectional area was smaller in the sonodynamic

therapy group than in the control, ultrasound, and PAD-S31 groups. It was concluded that sonodynamic therapy might be a feasible treatment modality for noninvasively inhibiting neointimal hyperplasia.

Anti-angiogenesis therapy is considered to be a new approach to various human cancers because angiogenesis is crucial for tumor growth (Emoto *et al.*, 2003). Moreover, ultrasound energy has been shown to enhance an anti tumor effect of a chemotherapeutic agent *in vitro* and *in vivo*. Uterine sarcoma is the most malignant neoplasm among the known uterine malignancies, which has a poor response to any chemotherapeutic agent currently used and also to radiotherapy. Our previous study (unpublished) showed anti tumor effect of TNP-470 (an analogue of fumagillin), an angiogenesis inhibitor, for human uterine sarcoma, *in vitro* and *in vivo*. This study firstly examined the therapeutic effect of angiogenesis inhibitor, combined with ultrasound irradiation for human cancer *in vivo*, and evaluated its vascularity real-time using a microbubble ultrasound contrast agent (Optison®). The uterine sarcoma xenografts were treated by 1 MHz ultrasound with an intensity of 2.0 W/cm^2 for 4 minutes three times per week, each after subcutaneous injection of TNP-470 at a dose of 30 mg/kg and this therapy was continued for eight weeks. The reduction of the volume, as well as the weight of the xenografts was significantly shown by this combined therapy, in comparison to a group of drug used alone or in the controls. No major side effect was observed in any mice of the groups. The effect of anti-angiogenesis for this tumor was demonstrated real-time by contrasted color ultrasound, non-invasively. The microvessel density of the tumors was significantly decreased in this combination therapy, compared with other groups. These results suggest that there is an accelerated (boosting) effect of ultrasound for anti-angiogenesis drug therapy for human uterine sarcoma, and this combination therapy might be a potential candidate for new cancer treatment.

Feril *et al.* (2005) recently reported monocytic leukemia cells (U937) killing effect by combining hyperthermia sensitive drug, 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) and exposure to nonthermal 1 MHz US for 1 minute at an intensity of 2.0 W/cm^2. Apoptosis measured by flow cytometry and free radical investigation using electron paramagnetic resonance (EPR) spin trapping, showed that US-induced cell lysis and apoptosis were enhanced in the presence of AAPH, regardless of the temperature

at the time of sonication. Although free radicals were increased in the combined treatment, this increase did not correlate well with cell killing. The mechanism of enhancement pointed to the increased uptake of the agent during sonication rather than potentiation by AAPH. Although much more research is needed to transfer experimental information to actual clinical cases, rapid advancement of HIFU (high intensity focused ultrasound) will act as an accelerating factor for future therapeutic strategies, combining anti cancer drug and ultrasound in patients (See Chap. VIII).

8. Molecular Imaging and Therapy

Molecular imaging is currently one of the most promising fields in medical research for the noninvasive assessment of physiologic and pathologic processes at the molecular level. Molecular imaging is not just an extension of the traditional process of image formation and interpretation, but is meant to improve diagnostic accuracy by providing an *in vivo* analog of immunocytochemistry or *in situ* hybridization. In this sense, molecular imaging is a revolutionary leap forward to diagnose a disease not just from morphological information, but by obtaining clues of the pathological malfunction of the lesion of interest at the molecular level. The object is to enhance the conspicuity of subtle pathologies by targeting the molecular components or processes that are the causes of disease. Recent advancements in nuclear imaging, ultrasound, MRI have initiated interest in molecular imaging across all modalities and across various medical fields, ranging from cancer to cardivascular areas. This new direction has only become possible because of the rapid progress in biotechnology (*e.g.*, the finalized human genome project, proteomics, and bioinformatics). Intense research and effort is currently being spent on identifying suitable molecular targets and preparing the specific sensitive site-targeted contrast agents. The use of ultrasound is undoubtedly a potential modality for molecular imaging.

The use of ultrasound has several characteristics that distinguishes it from the other molecular imaging modalities: (1) real time imaging is possible; (2) relatively short and efficient imaging protocols, compared with MRI or nuclear imaging; (3) non-invasiveness, minimal patient discomfort; (4) low operating costs; and lastly, (5) complementary therapeutic and imaging capabilities which neither MRI nor nuclear imaging can offer. The last item is by far the most outstanding characteristic. The major areas where

diagnostic ultrasound molecular imaging is being evaluated include angiogenesis (both endogenous and therapeutic), thrombus and cancer detection, identification of atherosclerotic plaque at risk for rupture and detection and quantification of markers of inflammation. In fact, therapeutic application is equally being considered in the same field as targeted drug delivery (See Chap. VI) and gene therapy (See Chap. III).

Although there are many strategies for designing microbubbles, there are mainly two types: passive/nonspecific targeting which relies on the properties of the shell and diameter of the bubble for accumulation in the vasculature; and active targeting, also known as specific targeting. The latter relies on adhesion ligands which makes it possible for the micro or nano bubbles to accumulate at a specific site. The current favorite molecular target for ultrasound is the expression of inflammation or immuno-associated molecules which can be visualized with molecular ultrasonography. Targeted contrast ultrasound has previously been utilized to detect vascular disease, including thrombi using fibrinogen targeted microbubbles (Unger *et al.*, 1998), inflammation using MBs targeted to P-selectin (Lindner *et al.*, 2001) or ICAM-1 (Villanueva *et al.*, 1998), and acute cardiac transplant rejection by targeting ICAM-1 (Weller *et al.*, 2003). Newly targeted microbubbles directed to the GPIIb IIIa receptor have been developed. These bioconjugate ligands were inserted into lipid-coated membranes of perfluorocarbon gas microbubbles and binding studies performed on activated platelets immobilized on cell culture plates. Targeted microbubble binding to clots in a flow through chamber was also being assessed. Microbubble binding studies on arteriolar and venular clots in a mouse cremasteric muscle model showed improved binding to vascular thrombi (Schumann *et al.*, 2002). Recent studies have shown the feasibility of using intravenously administered L-selectin ligand-specific polymer-stabilized air-filled microparticles for active targeting of peripheral lymph nodes under normal condition in animal models (Hauff *et al.*, 2004). This visualization technology can be applied as an indirect method of lymphography, thus making accurate identification of lymph nodes for biopsy and therapy possible. Endothelial cells of angiogenic tumor vasculature are characterized by altered expression of molecular markers on their surface. Numerous peptides have been identified that specifically bind tumor angiogenic endothelium, including the tripeptide arginine-arginine-leucine (RRL).

Weller *et al.* (2005) recently hypothesized that ultrasound contrast microbubbles targeted via linkage with RRL would specifically adhere to tumor angiogenic endothelium versus normal myocardium, and that this selective adhesion could be detected ultrasonically. Experimental results showed microbubble binding *in vitro* to tumor-derived cultured endothelium. Furthermore, *in vivo* ultrasonic detection of angiogenic tumor vasculature in a tumor-bearing mouse model demonstrated and showed that this technique could distinguish between normal tissue and tumor tissue.

Great advances have been reported in applying to various targets, including smaller microbubbles in the nanosize range which could function as indicators for ultrasound visualization. Targeted perfluorocarbon nanoparticles were the first reported molecular imaging agent for ultrasound applications and were shown to augment reflectivity from fibrin thrombi (Unger *et al.*, 1998). Additionally, targeting to vascular epitopes such as tissue factor, whose expression is induced in smooth muscle cells is possible, because these particles can penetrate through microfissures into the vascular media (Wickline *et al.*, 2003; Lanza *et al.*, 2003). Reflective microbubbles and liposomes have also been used to specifically target endothelial integrins (Dayton *et al.*, 2004; Leong-Poi *et al.*, 2003).

The ability to incorporate drugs or genes into detectable site-targeted nanosystems represents a new paradigm in therapeutics. Payloads of therapeutic agents, such as genes or radionuclides, can be complexed to the carriers themselves. Drugs can be linked to or dissolved within carrier lipid coatings, deposited in subsurface oil layers, or trapped within the carriers themselves. Drug delivery to specific cells from nanocarriers can occur by diffusion, particle fusion and internalization into cells, component (lipid-lipid) exchange and convective flux, biolistics, or some combination of these mechanisms. Nanoparticles are also useful for the delivery of pharmaceutical agents, after binding to target cellular epitopes by a mechanism known as "contact facilitated drug delivery". Binding and close apposition to the targeted cell membrane permits enhanced lipid-lipid exchange with the lipid monolayer of the nanoparticle, which accelerates convective flux of lipophilic drugs (*e.g.*, paclitaxel) dissolved in the outer lipid membrane of the nanoparticles into the targeted cells. The progress in molecular imaging by micro/nanobubbles will probably initiate further excitement for using this very technology for drug delivery. Ultrasound is technically the most

appropriate modality for this purpose, which will introduce new medical fields that are fusions between diagnostic and therapeutic.

9. Conclusions and Outlook

Research on the bioeffects of ultrasound alone and in the presence of various drugs to the patients has only just begun. Most investigations are still highly experimental and are far from being applicable in the clinical situation. However, such applications as HIFU therapy (see, Chap. VIII) for prostate cancer are already beginning to be widely used in patients as alternative non-operative modalities. Additionally, there exists an interesting biological phenomenon that cannot be ignored when non-thermal ultrasound is applied. The interaction between ultrasound and drugs can range from a change in the permeability of biological membrane to the manipulation of DNA into the cells. Recent discoveries have triggered the imagination of researchers in regenerative medicine and developmental research. Understanding the mechanism of micro/nano bubble collapse will eventually result in the optimization of the acoustics and the design of ultrasound devices for wider clinical therapeutic applications and research.

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References

Abe H, Kuroki M, Tachibana K, Li T, Awasthi A, Ueno A, Matsumoto H, Imakiire T, Yamauchi Y, Yamada H, Ariyoshi A, Kuroki M. *Anti Cancer Res* (2002) **22**(3): 1575–80.

Alexandrov AV, Demchuk AM, Hill MD. *Stroke* (2002) **33**: 354–55.

Alexandrov AV, Molina CA, Grotta JC, Garami Z, Ford SR, Alvarez-Sabin J, Montaner J, Saqqur M, Demchuk AM, Moye LA, Hill MD, Wojner AW. *N Engl J Med* (2004) **351**(21): 2170–8.

Arakawa K, Hagisawa K, Kusano H, Yoneyama S, Kurita A, Arai T, Kikuchi M, Sakata I, Umenura Si S, Ohsuzu F. *Circulation* (2002) **105**(2): 149–51.

Bao S, Thrall BD, Miller DL. *Ultrasound Med Biol* (1997) **23**(6): 953–9.

Blinc A, Francis CW, Trudnowski JL, Carstensen EL. *Blood* (1993) **81**(10): 2636–43

Culp WC, Porter TR, Lowery J, Xie F, Roberson PK, Marky L. *Stroke* (2004) **35**(10): 2407–11.

Daffertshofer M, Fatar M. *Eur J Ultrasound* (2002) **16**(1–2): 121–30.

Daffertshofer M, Hennerici M. *Lancet Neurol* (2003) **2**(5): 283–90.

Dayton PA, Pearson D, Clark J, Simon S, Schumann PA, Zutshi R, Matsunaga TO, Ferrara KW. *Mol Imaging* (2004) **3**(2): 125–34.

del Zoppo GJ, Higashida RT, Furlan AJ, Pessin MS, Rowley HA, Gent M. *Stroke* (1998) **29**(1): 4–11.

Emoto M, Ishiguro M, Iwasaki H, Kikuchi M, Kawarabayashi T. *Gynecol Oncol* (2003) **89**(1): 88–94.

Feril LB Jr, Kondo T, Zhao QL, Ogawa R, Tachibana K, Kudo N, Fujimoto S, Nakamura S. *Ultrasound Med Biol* (2003) **29**(2): 331–7.

Feril LB Jr, Ogawa R, Kobayashi H, Kikuchi H, Kondo T. *Ultrason Sonochem* (2005) **12**(6): 489–93.

Furuhata H, Kudo S. *Tokyo Jikeikai Med J* (1989) **104**: 1005–1012.

Francis CW, Onundarson PT, Carstensen EL, Blinc A, Meltzer RS, Schwarz K, Marder VJ. *J Clin Invest* (1992) **90**(5): 2063–8.

Greenleaf WJ, Bolander ME, Sarkar G, Goldring MB, Greenleaf JF. *Ultrasound Med Biol* (1998) **24**(4): 587–95.

Harrison GH, Balcer-Kubiczek EK, Eddy HA. *Int J Radiat Biol* (1991) **59**(6): 1453–66.

Hashiya N, Aoki M, Tachibana K, Taniyama Y, Yamasaki K, Hiraoka K, Makino H, Yasufumi K, Ogihara T, Morishita R. *Biochem Biophys Res Commun* (2004) **317**(2): 508–14.

Hauff P, Reinhardt M, Briel A, Debus N, Schirner M. *Radiology* (2004) **231**(3): 667–73.

Ishibashi T, Akiyama M, Onoue H, Abe T, Furuhata H. *Stroke* (2002) **33**(5): 1399–404.

Kimura M, Iijima S, Kobayashi K, Furuhata H. *Biol Pharm Bull* (1994) **17**(1): 126–30.

Koike H, Tomita N, Azuma H, Taniyama Y, Yamasaki K, Kunugiza Y, Tachibana K, Ogihara T, Morishita R. *J Gene Med* (2005) **7**(1): 108–16.

Kremkau FW, Kaufmann JS, Walker MM, Burch PG, Spurr CL. *Cancer* (1976) **37**(4): 1643–7.

Lanza GM, Wickline SA. *Curr Probl Cardiol* (2003) **28**(12): 625–53.
Leong-Poi H, Christiansen J, Klibanov AL, Kaul S, Lindner JR. *Circulation* (2003) **107**(3): 455–60.
Li T, Tachibana K, Kuroki M, Kuroki M. *Radiology* (2003) **229**(2): 423–8.
Lindner JR, Song J, Christiansen J, Klibanov AL, Xu F, Ley K. *Circulation* (2001) **104**(17): 2107–12.
Mahon BR, Nesbit GM, Barnwell SL, Clark W, Marotta TR, Weill A, Teal PA, Qureshi A. *AJNR Am J Neuroradiol* (2003) **24**(3): 534–8.
Manome Y, Nakamura M, Ohno T, Furuhata H. *Hum Gene Ther* (2000) 20;**11**(11): 1521–8.
Manome Y, Nakayama N, Nakayama K, Furuhata H. *Ultrasound Med Biol* (2005) **31**(5): 693–702.
Marmottant P, Hilgenfeldt S. *Nature* (2003) 8;**423**(6936): 153–6.
Marmottant P, Hilgenfeldt S. *Proc Natl Acad Sci USA* (2004) **101**(26): 9523–7.
Miura S, Tachibana K, Okamoto T, Saku K. *Biochem Biophys Res Commun* (2002) **298**(4): 587–90.
Mitragotri S. *Nat Rev Drug Discov* (2005) **4**(3): 255–60.
Miyoshi N, Misik V, Fukuda M, Riesz P. *Radiat Res* (1995) **143**(2): 194–202.
Mizushige K, Kondo I, Ohmori K, Hirao K, Matsuo H. *Ultrasound Med Biol* (1999) **25**(9): 1431–7.
Nakashima M, Tachibana K, Iohara K, Ito M, Ishikawa M, Akamine A. *Hum Gene Ther* (2003) **14**(6): 591–7.
Ogawa K, Tachibana K, Uchida T, Tai T, Yamashita N, Tsujita N, Miyauchi R. *Med Electron Microsc* (2001) **34**(4): 249–53.
Ogawa R, Kondo T, Honda H, Zhao QL, Fukuda S, Riesz P. *Ultrason Sonochem* (2002) **9**(4): 197–203.
Ohta S, Suzuki K, Tachibana K, Yamada G. *Genesis* (2003) **37**(2): 91–101.
Porter TR, LeVeen RF, Fox R, Kricsfeld A, Xie F. *Am Heart J* (1996) **132**(5): 964–8.
Porter TR, Hiser WL, Kricsfeld D, Deligonul U, Xie F, Iversen P, Radio S. *Ultrasound Med Biol* (2001) **27**(2): 259–65.
Postema M, van Wamel A, Lancee CT, de Jong N. *Ultrasound Med Biol* (2004) **30**(6): 827–40.
Pfaffenberger S, Devcic-Kuhar B, Kollmann C, Kastl SP, Kaun C, Speidl WS, Weiss TW, Demyanets S, Ullrich R, Sochor H, Wober C, Zeitlhofer J, Huber K, Groschl M, Benes E, Maurer G, Wojta J, Gottsauner-Wolf M. *Stroke* (2005) **36**(1): 124–8.
Saad AH, Hahn GM. *Cancer Res* (1989) **49**(21): 5931–4.

Schumann PA, Christiansen JP, Quigley RM, McCreery TP, Sweitzer RH, Unger EC, Lindner JR. *Matsunaga Invest Radiol* (2002) **37**(11): 587–93.

Tachibana S, Koga E. *Blood and Vessel* (1981) **12**: 450–53.

Tachibana K, Tachibana S. *J Pharm Pharmacol* (1991) **43**(4): 270–1.

Tachibana K. *J Vasc Interv Radiol* (1992) **3**(2): 299–303.

Tachibana K. *Pharm Res* (1992) **9**(7): 952–4.

Tachibana K, Tachibana S. *Circulation* (1995) **92**: 1148–1150.

Tachibana K, Uchida T, Hisano S, Morioka E. *Lancet* (1997) **349**(9048): 325.

Tachibana K, Uchida T, Ogawa K, Yamashita N, Tamura K. *Lancet* (1999) **353**(9162): 1409.

Taniyama Y, Tachibana K, Hiraoka K, Aoki M, Yamamoto S, Matsumoto K, Nakamura T, Ogihara T, Kaneda Y, Morishita R. *Gene Ther* (2002) **9**(6): 372–80.

Taniyama Y, Tachibana K, Hiraoka K, Namba T, Yamasaki K, Hashiya N, Aoki M, Ogihara T, Yasufumi K, Morishita R. *Circulation* (2002) **105**(10): 1233–9.

Uchida T, Tachibana K, Hisano S, Morioka E. *Anti cancer Drugs* (1997) **8**(4): 329–35.

Unger EC, McCreery TP, Sweitzer RH. *Invest Radiol* (1997) **32**(12): 723–7.

Unger EC, McCreery TP, Sweitzer RH, Shen D, Wu G. *Am J Cardiol* (1998) **81**(12A): 58G–61G.

Unger EC, Porter T, Culp W, Labell R, Matsunaga T, Zutshi R. *Adv Drug Deliv Rev* (2004) **56**(9): 1291–314.

Umemura S, Yumita N, Nishigaki R, Umemura K. *Jpn J Cancer Res* (1990) **81**(9): 962–6.

Villanueva FS, Jankowski RJ, Klibanov S, Pina ML, Alber SM, Watkins SC, Brandenburger GH, Wagner WR. *Circulation* (1998) **98**(1): 1–5.

Ward M, Wu J, Chiu JF. *J Acoust Soc Am* (1999) **105**(5): 2951–7.

Weller GE, Lu E, Csikari MM, Klibanov AL, Fischer D, Wagner WR, Villanueva FS. *Circulation* (2003) **108**(2): 218–24.

Weller GE, Wong MK, Modzelewski RA, Lu E, Klibanov AL, Wagner WR, Villanueva FS. *Cancer Res* (2005) **65**(2): 533–9.

Wickline SA, Lanza GM. *Circulation* (2003) **107**(8): 1092–5.

Yumita N, Sakata I, Nakajima S, Umemura S. *Biochim Biophys Acta* (2003) **1620**(1–3): 179–84.

ULTRASOUND IN MEDICINE: TO SEARCH AND DESTROY DISEASED TISSUES

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Ultrasound, defined as sound waves having frequencies too high (above 20 kHz) to be heard by human ear, is being used by some animals like dolphins and bats in navigation and hunting for preys. Such concept has been utilized in the Sound Navigating and Ranging or SONAR, which was discovered by Pierre Curie in the 1880s. In medicine, the ultrasonic era began in the 1940s when ultrasound was used to treat cancer. However, rapid progress in radiation therapy (using electromagnetic waves or subatomic particles) and chemotherapy (use of anticancer drugs) eventually overshadowed ultrasound as a treatment option against cancer. In the late 1940s, ultrasound was in the spot light again when it was introduced as a tool in medical imaging. Advances in piezoelectric materials (crystals that generate *electricity* when subjected to *mechanical stress*, or vice versa) used to manufacture ultrasound transducers have revolutionized ultrasound imaging from gray-scale to real-time imaging. While the recent advances in diagnostic ultrasound is not only rapid and finding wide use in all medical fields, other uses of ultrasound are continuously being unraveled in various scientific fields; producing outcomes useful to industries, households and even to ordinary individuals.

The expanding use of ultrasound led to more and wider research works on the mechanism by which ultrasound interacts with matter, especially on living cells and tissues. Equipped with modern technologies and more improved methodologies, medical researchers looked back to its potential for therapy. Studies on bioeffects [1-12], chemical effects [13] and thermal effects of ultrasound have revealed promising results. Application of ultrasound in therapy, particularly in the treatment of tumors [14], has recently been introduced, while on-going research works are geared for more future therapeutic applications. This review will summarize the current status of ultrasound in diagnosis (to search and identify the diseased tissues) and it's abilities to treat (destroy or cure) the diseased tissues.

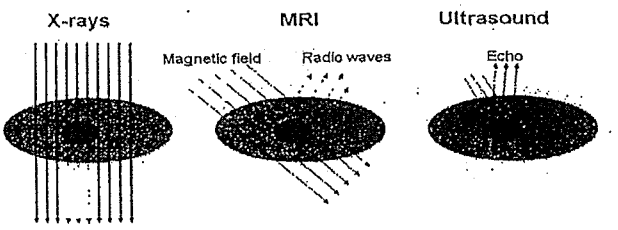

Fig. 1. Principles of medical imaging. X-rays have the ability to penetrate tissues of varying densities; MRI utilizes the ability of paramagnetic nuclei, such as that of H atoms in water molecules, to emit radio waves when subjected to a strong external magnetic field; while ultrasound does the imaging by capturing echoes from different tissues.

Ultrasound in diagnosis

The ability of ultrasound to penetrate soft tissue and produce echo (reflection) has made ultrasound a unique diagnostic imaging tool as compared to X-ray and magnetic resonance (MR) imaging (Fig. 1). The principle behind this is the ability of sound to produce an echo when it hits a certain object in varying magnitude, depending on the type of material. This is technically called echogenicity of

material. In the human body, different tissues have different characteristic responses to ultrasound. However, based on this principle alone, limitations do exist. Some tissues have similar echogenicity so that delineating them is difficult, and some structures are so small that the echo from larger structures overshadows them. One method to improve echo contrast is by making use of the Doppler effect of any moving part, such as the circulating blood. This principle utilizes the concept that moving object produces a different echo pattern with respect to a stationary one.

Recently, commercial development of echo contrast agents, such as microbubbles, has improved the efficiency of these ultrasonic imaging techniques.

Echo-contrast agents

Microbubbles (Fig. 1) are particularly useful because of the characteristics of any bubble to vibrate harmonically in response to ultrasound, thus, sending characteristic echoes, and hence the generic functional name, echo-contrast agents (ECA). Microbubbles generally localize within the vasculature during their lifetime, hence, providing a better echo-image of the vasculature and a good contrast between tissues with different levels of vascularization. This aspect is particularly important in the diagnosis of tumor tissues that have particular vascularization patterns.

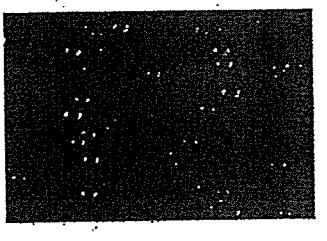

Fig. 2. Microbubbles. Micrograph of microbubbles at 200x magnification. White bar represents a 10 micrometer length.

Ultrasound in therapy

Ultrasound was said to stimulate growth of cells in tissues that requires growth, repair or healing. At higher intensities, however, ultrasound can induce cell and tissue damage and other biological effects [15]. All these biological effects of ultrasound were studied for possible application in therapy. Very high intensity ultrasound can be focused into a target tissue, e.g. tumor, by generating lethal temperatures. This is technically called tumor ablation by high-intensity focused ultrasound (HIFU) [16].

High-Intensity Focused Ultrasound (HIFU)

The principle of HIFU is simple Ultrasound transducers can be constructed t focus sound and deposit acoustic energy int a concentrated volume, just as a magnifyin glass can focus light and deposit energy into concentrated volume. In tumor ablation, suc high energy focus creates a discree predictable and controlled ablation of th target tissue, minimizing the application energy to unintended tissue (Fig. 3).

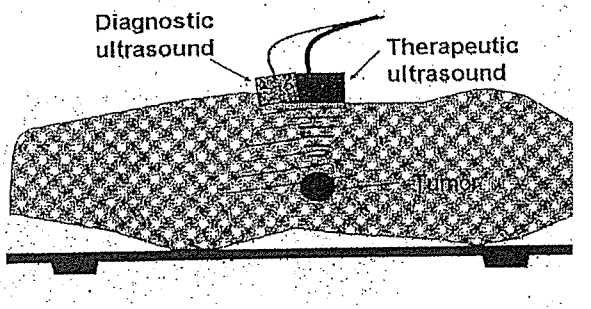

Fig. 3. Use of diagnostic ultrasound to visualize tum during thermal ablation with high intensity focus ultrasound (HIFU), and also to monitor the therapeu response.

Such procedure is considered nc invasive (without cutting or opening the boc as compared to surgery where the body is (or opened) to remove the tumor. Nc invasive method avoids complications fr the procedure, reduces the cost of medi

care, and results to less suffering to the patient.

Hyperthermia

Hyperthermia, temperatures ranging from 40 to 44°C, has long been recognized as a modality in cancer therapy [17-19]. The principle is based on the observation that cancer cells are more prone to die with these temperatures than the normal cells in the human body.

There were various methods used to generate heat to attain the desired temperature. More recently, ultrasound-generated heat was found to be more effective than those generated by other methods.

Non-thermal biological effects of ultrasound

We have confirmed previous findings that ultrasound can induce cell killing even without significant temperature rise and even at very low intensities. Such biological effects are usually cavitations related (Fig. 4). Some factors that enhance these effects and factors that inhibit them were identified and characterized [4].

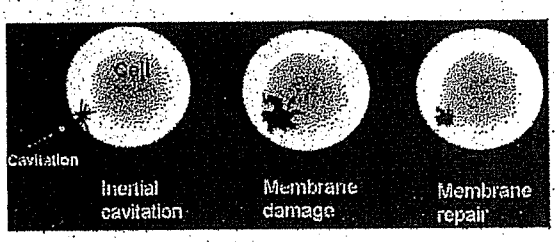

Fig. 4. Cell membrane damage caused by cavitation formed by sonication, and the cellular repair.

Nonthermal ultrasound enhances Hyperthermia induced apoptosis

Hyperthermia induces apoptosis and is being used in medicine to treat tumors. A study shows that the hyperthermia-induced apoptosis can be enhanced by ultrasound at intensities even below threshold for cell killing. Since ultrasound is currently being used to generate heat for hyperthermia therapy, this data help explain why ultrasound was shown to be more effective than other technology used to generate hyperthermia [12].

Echo-contrast agents enhance ultrasound-induced cell killing and DNA transfection

Different ECAs were used (Levovist, Optison and YM454) in a study [11]. The result showed that Levovist is effective in enhancing ultrasound-mediated gene transfection, while in a different study it was shown that Optison and YM454 are effective in enhancing cell killing. The mechanism should include transient pore formation. The main factors involved are the stability and size of the microbubbles as these are responsible in creating inertial cavitation (acoustically generated oscillation and collapse of bubbles).

Ultrasound-induced cell killing enhanced by some agent

Synergistic effect between ultrasound and some agents, especially anticancer drugs, were observed [14]. In one of the studies a temperature dependent free radical generator, AAPH, was used in combination with ultrasound [20]. Cell killing was enhanced and free radical generated by AAPH was increased by sonication. Data also indicated that increased uptake of the agent rather than the increased free radical production was responsible in the enhancement.

Hypotonia enhances ultrasound-induced cell killing

Hypotonia (146 mOsm) can induce nonlethal swelling of cells. This osmotic cell

swelling was found to enhance ultrasound-induced cell killing [1]. Although low viscosity can modify acoustic cavitation formation, the data showed that it did not play an important role in the enhancement but rather the mechanical effect of radiation force on the swollen cells. As described by Nyborg (1968) that a force F towards the direction x, acting on a particle of volume v in a liquid medium where radiation pressure (as in acoustic streaming and microstreaming) is applied, F is given by

$$F=v\,(1-\beta)\partial T(\partial x)^{-1}$$

where T is the time-averaged volume density of kinetic energy, while $\beta=\rho o/\rho$, is the density ratio. Here, ρo is the density of the medium, and ρ is the density of the particle (cell).

Addition of hypotonic medium into the cells will result to a decrease in β value, thus increasing the value F. This is followed by cell volume increase which will result to a directly proportional increase of the value F. This would mean that a 3 x increase in cell volume (at about 5 min of hypotonia), would result to more than 3 x increase in magnitude for F when combined with the density effect.

Ultrasound-induced cell killing is inhibited by carbon dioxide

Dose dependent inhibition of ultrasound effects (both bioeffects and chemical effects such as free radical production) was observed when equal doses of HCl and H_2CO_3 were used to generated measurable concentration of CO_2 in the medium used [10]. It is known that CO_2 lowers the final temperature of collapsing bubbles (Fig. 5) since it has a lower value of $\gamma=C_p/C_v$. For the adiabatic collapse of a cavitation bubble, the final intracavity temperature at the end of the collapse, T_f is given by

$$T_f = T_i(R_{max}/R_{min})^{3(\gamma-1)}$$

where T_i is the initial temperature, γ is the specific heat ratio (C_p/C_v) of the gas inside the bubble; R_{max} is the initial radius of a bubble which collapses to a final radius of R_{min}.

This finding implies how handling of cell samples is important in experiments related to ultrasound effects [2], whch also guides researches to consider CO_2 concentration in the living body when doing in vivo studies.

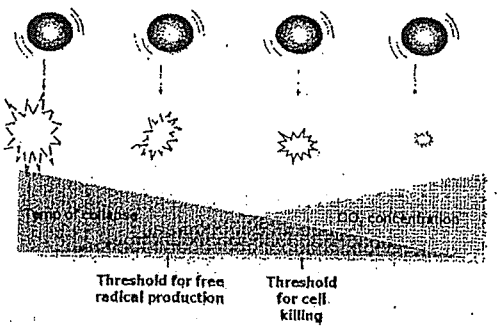

Fig. 5. Effect of carbon dioxide on the cavitational activity resulting to changes in the ultrasound-induced cell killing and free radical production.

Related studies

Based on the above findings on the mechanism of cell killing induced by ultrasound, it is hypothesized that certain conditions would optimize killing on a desired mode of cell death, e.g. apoptosis. Recent findings have confirmed such hypothesis on apoptosis [3,9], and have also lead to an improved ultrasound-mediated gene transfections [21-23].

In summary, the widening use of ultrasound in therapy and the sophistication of ultrasound imaging, suggest that combination of diagnostic and therapeutic ultrasound would further lead to a more advanced and more effective management of disease, particularly cancers.

References

1. **Feril LB Jr**, Kondo T, Takaya K, Riesz P. Enhanced ultrasound-induced apoptosis and cell lysis by a hypotonic medium. *International Journal of Radiation Biology*, 2004; **80**, 165-175.
2. **Feril LB Jr**, Kondo T. Major factors involved in the inhibition of ultrasound-induced free radical production and cell killing by pre-sonication incubations and by high cell density. *Ultrasonics Sonochemistry* 2005; **12**, 353-357.
3. **Feril LB Jr,** Kondo T, Cui Z-G, Tabuchi Y, Zhao Q-L, Hidetaka Ando, Takuro Misaki, Yoshikawa H, Umemura S. Apoptosis induced by the sonomechanical effects of low intensity pulsed ultrasound in a human leukemia cell line. *Cancer Letters* 2005; **221**:145-152.
4. **Feril LB Jr,** Kondo T. Biological effects of low intensity ultrasound: the mechanism involved, and its implications on therapy and on biosafety of ultrasound. *Journal of Radiation Research* 2004; **45**, 479-489.
5. Honda H, Kondo T, Zhao QL, **Feril LB Jr,** Kitagawa H. Role of intracellular calcium ions and reactive oxygen species in apoptosis induced by ultrasound. *Ultrasound in Medicine and Biology* 2004, **30**:683-92.
6. Kagiya G, Ogawa R, Tabuchi Y, **Feril LB Jr,** Nozaki T, Fukuda S, Yamamoto K and Kondo T. Expression of heme oxygenase-1 due to intracellular reactive oxygen species induced by ultrasound. *Ultrasonics Sonochemistry* (in press)
7. Kagiya G, Tabuchi Y, **Feril LB Jr**, Ogawa O, Zhao Q-L, Kudo N, Hiraoka W, **Tachibana K**, Umemura S, and Kondo T. Confirmation of enhanced expression of heme oxygenase-1 gene induced by ultrasound and its mechanism: Analysis by cDNA microarray system, real-time quantitative PCR, and western blotting. *Journal of Medical Ultrasonics* (in press)
8. Hiraoka W, Honda H, **Feril LB Jr**, Kudo N, Kondo T. Comparison between sonodynamic effect and photodynamic effect with photosensitizers on free radical formation and cell killing *Ultrasonics Sonochemistry* (in press)
9. Ando H, **Feril LB Jr**, Kondo T, Tabuchi Y, Ogawa R, Zhao QL, Cui ZG, Umemura S, Yoshikawa H and Misaki T. A echo-contrast agent, Levovist, lowers the ultrasound intensity required to induce apoptosis of human leukemia cells. *Cancer Letters* (in press)
10. **Feril LB Jr**, Kondo T, Ogawa R, Zhao QL. Dose-dependent inhibition of ultrasound-induced cell killing and free radical production by carbon dioxide. *Ultrasonics Sonochemistry* 2003; **10**: 81-4.
11. **Feril LB Jr**, Kondo T, Zhao QL, Ogawa R, **Tachibana K**, Kudo N, Fujimoto S, Nakamura S. Enhancement of ultrasound-induced apoptosis and cell lysis by echo-contrast agents. *Ultrasound in Medicine and Biology* 2003; **29**: 331-7.
12. **Feril LB Jr**, Kondo T, Zhao QL, Ogawa R. Enhancement of hyperthermia-induced apoptosis by non-thermal effects of ultrasound. *Cancer Letters* 2002; **178**: 63-70.
13. **Feril, L.B. Jr.**, Kondo, T Ultrasound liberates nitric oxide (NO) from the caged NO compound *N,N'*-bis(carboxymethyl)-*N,N'*-dinitroso-p-phenylenediamine sodium salt. *Ultrasonics Sonochemistry* (in press)
14. **Feril LB Jr**, Kondo T, Umemura S, **Tachibana K**, Manalo AH, Riesz P. Sound waves and antineoplastic drugs: the possibility of an enhanced combined anticancer therapy. *Journal of Medical Ultrasonics* 2002; **29**: 173-187.
15. **Tachibana K**, Uchida T, Ogawa K, Yamashita N, Tamura K. Induction of

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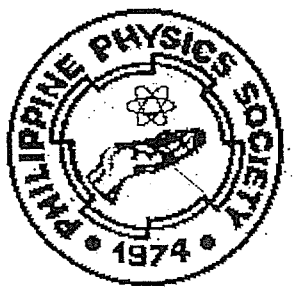

USING ULTRASOUND FOR DRUG DELIVERY

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Therapeutic ultrasound has mainly been applied for its thermal or mechanical effects. Applications of high-energy ultrasound to ablate cancers are now in clinical trials. Recently, there have been numerous reports on the application of non-thermal ultrasound in treating various diseases, especially in combination with drugs. Furthermore, the introduction of microbubbles and nanobubbles as a carrier/enhancer of drugs has added a whole new dimension in therapeutic ultrasound. In the past decade, progress in the pharmaceutical side has further added excitement in applying this technology especially in the fields of molecular biology, gene therapy and regenerative medicine. Alternatively from the device side, therapeutic ultrasound catheters and extracorporeal ultrasound probes are under development specifically for this purpose, some are already in the clinical trials. Such examples as enhancement of thrombolytic agents by ultrasound have proven to be beneficial to patients with acute stroke and peripheral arterial occlusions. Non-invasive focused ultrasound in conjunction with anti-cancer drugs may help to reduce tumor size, lessen recurrence, as well as reduce severe drug side effects. Chemical activation of drugs by ultrasound energy for treatment of atherosclerosis and tumors is another new field recently termed as "Sonodynamic Therapy". Lastly, advances in molecular imaging have also initiated great expectations in applying ultrasound for both diagnosis and therapy at the same time. Microbubbles or nanobubbles targeted at the molecular level will permit medical doctors to make a final diagnosis of a disease by ultrasound and immediately proceed to therapeutic ultrasound. This chapter will put emphasis on emerging technologies in therapeutic ultrasound related to the above topics.

Introduction

Thermal effects of ultrasound are currently applied in therapy. A completely new concept of using non-thermal ultrasound has broadened the scope of therapeutic ultrasound. Non-thermal mechanisms include various forms of energy such as those by cavitation, acoustic streaming, micro jets formation, and radiation force which could increase bioavailability of drugs to target tissues, enhancing its effects. Low energy ultrasound alone produces minimal damage to the tissue but augments the bioeffects of drugs leading to a more beneficial outcome.

There are thousands of types of drugs available in the market today. A greater number of drugs never reached the market for reasons including toxicity and side effects, particularly agents for cancer chemotherapy. Such agents when administered systemically can kill solid tumors but at the same time can severely damage healthy tissues and organs. Renal, heart failure and liver dysfunction are not rare among cancer patients treated with these highly toxic chemotherapeutic agents. Obviously, a tumor site-specific drug is desired in this case. Drugs for hypertension require a maintained concentration level in order to attain stable blood pressure, while on the contrary, intermittent insulin injection is needed after each meal for diabetic patients. A

new drug release system which could eliminate cumbersome administration is therefore targeted. These issues have been a major challenge to pharmacologists for many years. Pharmaceutical companies have attempted to address these problems by suggesting the concept or strategy termed "drug delivery system (DDS)" since the 70's. The two keywords for DDS are "targeting" and "controlled release". The major goal was to avoid severe side effects by reducing the total dosage but at the same time concentrating the drug at the target site. Additionally, controlled drug release permits more control over the amount of drug administered in a certain time frame. Recent progress in the application of ultrasound energy for DDS has demonstrated it to be a promising modality for both "targeting" and "controlled release" of drugs.

The application of nonthermal ultrasound for DDS can be classified into three major categories. First, ultrasound energy can actually help agents penetrate through various tissues. It has been demonstrated that acoustic pressure can "push" materials into the skin, blood clots or other tissues. Secondly, ultrasound can have a direct effect on the membrane and change the permeability or absorption of the drug into cells and tissues. Lastly, ultrasound can "release" drugs from a certain drug carrier or change the chemical properties of the drug itself at localized site. Microbubbles and nanobubbles can carry and release a certain drug at specific target site at a particular time by ultrasound. Ultrasound-sensitive agents such as hematoporphyrin, a nontoxic agent, can also be activated by ultrasound at a localized lesion and can thus result in an effective killing of cancer cells. A wide variety of applications for drug delivery with ultrasound are currently under investigation in many fields. The most futuristic investigation under progress is the use of ultrasound for gene therapy. The DNA can be injected through the cell membrane as if a "micro syringe" and induce transfection with ultrasound energy. Induction of gene transfer by ultrasound to the cell could result in regeneration of blood vessels, nerves or any other tissue. With the help of microbubbles or nanobubbles, it is possible to enhance cell membrane permeability to drugs. Recent findings on the use of ultrasound in gene therapy suggest that this technology could prove critical in the emergence of "next generation" drugs in the clinical arena.

Gene Transfection

Genes are the basic physical and functional units of heredity, any defect in it could lead to disease. Gene therapy is a technique for correcting defective genes responsible in a disease. Researchers are currently developing several approaches for correcting faulty genes to cure various diseases. A normal gene may be inserted to replace a nonfunctional gene. The current system of using viruses as the gene carrier into the target lesion presents a variety of potential problems to the patient; toxicity, immune and inflammatory responses, and gene control. Greenleaf (1998) first approached these problems by way of using non-viral plasmid DNA in combination with ultrasound. It was postulated that ultrasound would initiate the delivery of DNA through the cellular membrane to the cells. Bao (1997) further added microbubbles to increase the rate of DNA transfer. Ward (1999) and Tachibana (1999) had earlier theorized that liquid microjects induced in the event of collapse of microbubbles could be the mechanism in which DNA easily penetrates the cell membrane. Scanning electron microscopy and high-speed video imaging technologies have recently revealed images of collapsing microbubbles and ruptured cell membrane surface that supports this theory. Unger (2004) introduced the concept of "tailored made" microbubbles and

nanobubbles that target specific tissue lesions to deliver drugs and DNA.

Stroke Therapy

The Stroke is the third most common cause of death in the United States. Stroke is caused by an interruption of the flow of blood to the brain (an ischemic stroke) or the rupture of blood vessels in the brain (a hemorrhagic stroke), which in turn causes brain cells in the affected area to die. The thrombolytic agent, tissue plasminogen activator (t-PA) is the most effective FDA approved drug to treat ischemic stroke; however, the success rate is low. In general, higher dosages of lytics increase the treatment success rate but have also resulted in higher incidence of side effects such as unwanted bleeding in the brain and the digestive system.

It is well known that the thrombus structure resembles a fibrin net, with considerable space between the fibrin and red cells. Transport of fibrinolytic drugs into the thrombus is an important determination of the clot lysis rate. However, in the early stages of thrombolytic therapy, only a fraction of therapeutically administered plasmingogen activators can penetrate into the clots by passive diffusion. Investigators have shown that ultrasound accelerates fibrinolysis. Though the exact mechanisms are unknown, it is theorized that non-ultrasound alters the surface of the thrombus affecting its interaction with the agent. Ultrasound itself does not seem to activate the fibrinolytic cascade. Blinc (1993) demonstrated that ultrasound energy did not accelerate the hydrolysis of a peptide substrate by rt-PA, and the rate of plasmin degradation of fibrinogen was not increased. Acceleration of fibrinolysis by ultrasound also required the presence of a fibrin gel and was seen with clots of whole blood, plasma, and purified fibrin. Kimura (1994) confirmed the increase of clot lysis and the fibrin degradation product, D-dimer, after ultrasound exposure plus re-PA. These data support the theory that the accelerated fibrinolysis by ultrasound is primarily due to an enhanced drug transport within the clots through a non-thermal related mechanism. The most likely mechanism in which ultrasound provokes drug movement into the thrombus is acoustic cavitation, which can be termed to as the formation and collapse of bubbles in liquids, which can generate high velocity cavitation related phenomena and can assist drug diffusion, especially at locations where acoustic impedance differs. Tachibana et al (1995) reported further acceleration of fibrinolysis by ultrasound in the presence of albumin microbubbles around the clots. These microbubbles were originally designed for diagnostic echo contrast, however, when they were exposed to more intense ultrasound in this case, it was postulated that this material served as a nuclei for cavitation induction, thus resulting in more fibrinolysis.

Tachibana (1992) demonstrated in vitro that relatively low-intensity ultrasound irradiation of clots in the presence of lytic agents can reduce the amount of drug required by one-tenth and shorten the lysis duration to one-fifth of the original time. This has also been confirmed by other researchers (Francis 1992, Blinc 1993). Although the minimum ultrasound intensity needed to induce acceleration of fibrinolysis is currently under discussion, recent reports have shown that relatively low mechanical index (MI) energy ranging from 0.1 to 1.0 W/cm^2 can produce enhanced fibrinolytic effects. Nonthermal effects of ultrasound contribute to the penetration of drugs into the thrombus. Experiments under conditions where cavitation is more easily produced have resulted in further enhancement of thrombolysis. Increased thrombolysis may also be associated with the unidirectional motion of a fluid or drug known as acoustic streaming, which originates within close range of the ultrasound transducers.

Researchers have studied the driving force of acoustic streaming of microparticles theoretically and experimentally in various fluid conditions. Another possible explanation for the increased thrombolysis may be the temporary effect of ultrasound on the thrombus itself. It was suggested that bubble formation, growth and collapse causes reversible alteration in the fibrin structure that may result in an increased flow of the drug into the thrombus.

Fig. 1. Catheter type therapeutic ultrasound device that could be inserted into the middle cerbral artery in humans

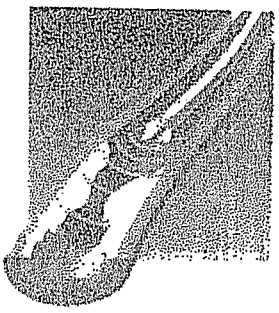
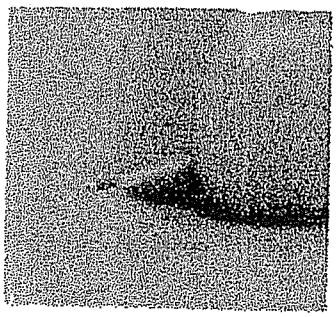


Fig. 2. The MicroLysUS catheter (EKOS Corporation)



Clinical application of this new therapeutic ultrasound method has already started since 2001. Clinical trials in Europe and in the USA have been reported using miniature ultrasound transducers at the tip of catheters that approach the clots via arterial vessels (MicroLysUS infusion catheter, EKOS Corp, USA). The lytic drug, urokinase, is released at the distal end of the catheter during ultrasound irradiation (Fig. 1 and 2). The major goal of the catheters used in this study was to apply ultrasound at shorter distances with smaller ultrasound probes, and, at the same time minimize damage to surrounding normal tissues. Mahon (2003) presented early experience with the MicroLysUS infusion catheter for acute embolic stroke treatment in North America. This study was designed to demonstrate the safety of the device and to determine if ultrasound accelerates thrombolysis and improves clinical outcomes. The EKOS catheter for leg peripheral arterial thrombolysis was approved by the FDA in 2004 and will probably be the first drug/ ultrasound combination product to hit the market in the cardiovascular field.

Ishibashi (2002) investigated an alternative way of sonicating transcranially to accelerate thrombolysis. A noninvasive method was tested in an occlusion model of rabbit femoral artery, produced with thrombin after establishment of stenotic flow and endothelial damage. After stable occlusion was confirmed, monteplase (mtPA) was administered intravenously, and ultrasound (490 kHz, 0.13 W/cm^2) was applied through a piece of temporal bone (TUS group). The recanalization ratio in the TUS group was higher than that in the tPA group. Pfaffenberger et al modified this experiment to determine if 1.8-MHz commercial diagnostic ultrasound devices would accelerate thrombolysis. Duplex-Doppler, continuous wave-Doppler, and pulsed wave (PW)-Doppler were compared on their impact on recombinant tissue plasminogen activator (rtPA) mediated thrombolysis. Blood clots were transtemporally sonicated in a human stroke model. Furthermore, without temporal bone, PW-Doppler plus rtPA showed a significant enhancement in relative clot weight loss. Ultrasound attenuation measurements revealed decreases of the output intensity of over 85%, depending on temporal bone thickness. Ultrasound attenuation of the bone is a major limiting factor in the case of high frequency

transcranial ultrasound application. Clinical investigations were aggressively carried out by Alexandrov (2002) with diagnostic transcranial ultrasound. The initial study included 40 acute stroke patients with occlusions of the middle cerebral artery (MCA), internal carotid artery, or basilar artery, which revealed high rates of complete recanalization with dramatic clinical recovery when continuous transcranial Doppler (TCD) monitoring was used during tissue plasminogen activator (tPA) infusion. Later, data of the 126 randomized phase II Combined Lysis of Thrombus in Brain Ischemia Using Transcranial Ultrasound and Systemic tPA (CLOTBUST) trial was published (Alexandrov 2002), providing clinical evidence for the existence of ultrasound-enhanced thrombolysis in the middle cerebral artery thrombus. Large clinical trials concluded that continuous transcranial Doppler augmented t-PA-induced arterial recanalization, with a non-significant trend toward an increased rate of recovery from stroke, as compared with placebo. More evaluation is needed to see if diagnostic level ultrasound intensity can truly penetrate the skull and accelerate thrombolysis.

To summarize, the differences between external and internal ultrasound applications are: 1) in external application, relatively higher energy and perhaps lower frequency ultrasound is needed at the surface of the body to sufficiently deliver energy to deeply located thrombus, 2) ultrasound must propagate through the skull and this prevents sufficient ultrasound energy from reaching the target accurately. 3) For internal catheter ultrasound, the number of hospitals that can actually conduct this treatment within 3 to 6 hours after onset of stroke is limited. More clinical studies are needed to evaluate the medical significance of accelerated thrombolysis by ultrasound energy. However, this therapeutic ultrasound application for stroke seems to be the most promising among various ultrasound drug deliveries in clinical trials known to date.

Microbubbles

Microbodies of air or a gas, suspended in a liquid are exceptionally efficient ultrasound reflectors for echography, hence are useful as ultrasonic contrast agents. For instance, injecting into the bloodstream of living bodies suspensions of engineered microbubbles (in the range of 0.5 to 10 μm in diameter) in a carrier liquid will strongly reinforce ultrasonic echographic imaging, thus aiding the visualization of internal organs, for the detection of cardiovascular diseases. Coated microbubbles have the advantage of being stable in the body for a significant period of time, as the shells serve to protect the gases of the microbubbles from diffusion into the bloodstream. Second-generation microbubbles contain perfluorocarbon gas rather than air, which results in an even longer life span of contrast agents within the circulatory system. This permits a longer window time for the echographers to observe patients. Recently, various in vitro and in vivo experiment have demonstrated that echo contrast agent microbubbles can be intentionally ruptured by diagnostic and therapeutic ultrasound. This acoustically induced destruction and collapse of the microbubbles produces a high amplitude response. Violent microstreaming can be produced during microbubble collapse. Researchers have hypothesized that these microjets or microstreaming could be applied to promote diffusion of drugs into various tissues and lesions. Albumin microbubbles were first used in conjunction with ultrasound to further enhance the effects of thrombolytic agents (Tachibana 1995).

Porter (1996) later reported that intravenous perfluorocarbon-exposed sonicated dextrose albumin (PESDA) microbubbles in the presence of low