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Contrast Enhanced Ultrasound: An Overview

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A B S T R A C T

Application of ultrasound contrast medium to traditional medical sonography is known as Contrast-enhanced ultrasound (CEUS) that enhances contrast during ultrasnography by increasing ultrasound backscatter (reflection) of the ultrasound waves. Contrast-enhanced ultrasound can be used in diagnostic imaging, organ edge delineation, echocardiography, blood volume perfusion, lesion characterization, drug or gene delivery, molecular imaging etc. Different types of contrast media used in ultrasonography are agitated saline, microbubbles, nanobubbles etc. Adverse reactions of contrast agent include headache, hypersensitivity abdominal pain, diarrhoea, dyspepsia, hypertension, leg cramps etc. Although CEUS is popular now a days but with certain limitations such as more heat production with increase in frequency, short life of microbubbles, continuous monitoring, occasional microvasculature rupture and haemolysis. In conclusion, CEUS is an advanced technique for absolute quantification of tissue perfusion, drugs and genes delivery, differential diagnosis and monitoring therapy response.

Introduction

Contrast-enhanced ultrasound (CEUS) is the application of ultrasound contrast medium to traditional medical sonography. Ultrasound contrast agents rely on the different ways in which sound waves are reflected from interfaces between substances. This may be the surface of a small air bubble or a more complex structure (https://en.wikipedia.org/wiki/Contrast-enhanced_ultrasound).

Commercially available contrast media are gas-filled microbubbles that are administered

intravenously to the systemic circulation. Microbubbles have a high degree of echogenicity (the ability of an object to reflect ultrasound waves). There is a great difference in echogenicity between the gas in the microbubble and the soft tissue surroundings of body. Thus, ultrasonic imaging using microbubble contrast agents enhances the ultrasound backscatter (reflection) of the ultrasound waves, to produce a sonogram with increased contrast due to high echogenicity difference. Contrast-enhanced ultrasound can be used to image blood perfusion in organs, measure blood flow rate in heart and other organs and for other applications. CEUS is an option and was shown to improve cancer detection and tumor characterization, decreasing the number of biopsies, or during surgery in brain cancer patients (Kitano *et al.,* 2012; Uemura *et al.*, 2013; Prada *et al.,* 2014).

History

Application of CEUS started in late 1960s when a detectable signal change during US examination was observed after injection of agitated saline caused (Kremkau *et al.,* 1970). Contrast enhancement by the compressible gas core of saline bubbles was caused by backscatter of US wave. But, due to the high surface tension saline bubbles were unstable. Kremkau *et al.,* 1970 formed more stable bubbles by injection of autologous blood at adequately rapid rates. Nonetheless, bubbles still lacked sufficient lifetime and a defined size. After more than 20 years first stable commercially available and FDA approved USCA- Albunex, an albumin-coated and air-filled microsphere was found (Feinstein *et al.,* 1990).

Since then, stability and biocompatibility of USCA have been continuously improved and bubbles have been modified to specifically target certain surface molecules expressed in pathological alterations. Apart from their support for imaging and diagnostics, micro- and NBs are object of increased interest for therapeutic applications too. Recent studies used the disrupting effect of MB-enhanced US on the BBB in combination with transplantation of mesenchymal stem cells for treatment of brain ischemia, or used MBs as carriers of drugs, siRNA and mRNA (Dewitte *et al.,* 2014; Gong *et al.,* 2014). This broad field of different uses makes USCA attractive for research and beneficial for patients.

Currently three different MB-based CA are clinically approved in the United States/North America and Europe, and a fourth is clinically used in Japan and South Korea, but the variety among the investigative CA is much broader and frequently produces new, promising progenies. Since examinations with those approved CA are common in the clinics, guidelines for CEUS imaging of the liver exist to guarantee proper and comparable examinations and an improvement for diagnosis and therapy (Claudon *et al.,* 2013).

Principle of CEUS

US contrast agents consist of microbubbles containing air or various gases within a shell. When a US contrast agent is administered into the vasculature, it enhances the backscatter of ultrasound waves by resonance within sonic windows (Sontum, 2008). This results in a marked amplification of the signals from the blood flow and provides additional information about the microvasculature (Gries, 2004).

Types of contrast media

First generation

First-generation ultrasound contrast agents contained microbubbles of air that were dissolved in blood when exposed to acoustic pressure in the ultrasound field. They were

therefore present in the bloodstream for a limited time.

Second generation

Second-generation contrast agents include microbubbles of perfluorocarbon, nitrogen gas or sulfur hexafluoride stabilized in a phospholipid membrane. When exposed to the ultrasound beam, bubbles oscillate (they are being compressed by the effect of positive pressure created by the ultrasound waves and they expand in the negative pressure phase). The compression of the gas is greater than expansion which creates a non-linear response (echo). This greatly affects ultrasound backscatter and increases vascular contrast in a similar manner to intravenous contrast media used in CT and MRI (Balint Botz *et al.*,)

Microbubble

As the name suggests, the diameter ranges between 1 and 10 µm. This size normally limits the application of MB to the intravascular system to assess functional parameters like vascularity, perfusion, blood flow velocity, angiogenicity or to characterize vasculature molecularly by using targeted MB (Yuan and Rychak, 2013). Hence, shell was introduced to produce soft- and hard-shell bubbles, that provide multiple specific applications of MB by changing their visco-elastic properties (Hoff *et al.*, 1996; *Kiessling et al.*, 2014). To rule out possible changes in bubble-size by air diffusing along the concentration gradient between blood and bubble, MB is produced with a defined mixture of PFC and air, so that Laplace and arterial pressure are in equilibrium (Schutt *et al.*, 1996).

MBs of a diameter below the maximum of 10 µm were obtainable and for certain mixtures a shelf time of several weeks and a high echogenicity in B-mode US imaging were shown (Singhal *et al.*, 1993; Wheatley *et al.*, 1994). Still, those MB that succeeded in clinical trials were of different materials. Imagent® (IMCOR Pharmaceuticals Inc., San Diego, CA, USA) MB of approximately 5 µm in diameter, filled with a mixture of air and PFCs, were first tested for renal and liver perfusion studies in rabbits, later for myocardial perfusion and detection of general blood flow abnormalities using Doppler US. It showed promising contrast and compatibility with almost no adverse side effects in first clinical trials (Taylor *et al.*, 1996; Sirlin *et al.*, 1997; Pelura, 1998).

Different types of microbubbles used in ultrasonography are

Polymer-shelled microbubbles

First polymers used for US applications were naturally occurring air-filled polymers. Gelatin was among the first biopolymers to be tested, but the production of adequately small MB turned out to be difficult and their circulation time was short (Carroll *et al.*, 1980). Cyanoacrylate polymers were first used as a shell material by Fritzsch *et al.*, (1994).

Hard-shell microbubbles

They mainly consists of gas bubbles with a coating of lower visco-elastical properties such as polymers or denatured proteins, as well as porous silica materials encapsulating gas.

Protein-shelled microbubbles

Though protein-shelled microbubbles are less resistant to US waves than polymer-coated MB, but with a longer history of use and development, the first commercially available USCA was the protein-shelled MB Albunex® (Molecular Biosystems, San Diego, CA, USA).

First animal experiments showed an enhanced contrast in 2D echocardiography after intravenous injection (Keller *et al.,* 1987) and a behavior similar to erythrocytes to guarantee no interferences in coronary flow or hemodynamics caused by the CA during myocardial US examination (Keller *et al.,* 1988, 1989).

Soft-shell microbubbles

Soft-shell MBs are commonly used for examinations using a low mechanical index (MI) since these MB are sensitively detectable by their non-linear oscillations. The better oscillation properties of SS-MB compared with HS-MB are due to the thinner, more flexible shells, which are held together not by covalent bonding, but hydrophobic interactions. Therefore, after slight shell disruptions, the shell seals itself to minimize surface tension (Borden *et al.,* 2005; Brismar *et al.,* 2012). Most common shell materials for SS-MB are surfactant molecules or phospholipids, where the length of the acyl chain mainly influences the bubbles' acoustic dissolution and the monolayers' cohesiveness (Borden *et al.,* 2005).

Phospholipid microbubbles

The first lipid-based USCA that made it to clinical trials and the clinics was Perflutren, sold as Definity or Luminity (Lantheus Medical Imaging, North Billerica, MA, USA). It contains perfluoropropane-filled MB in a shell made of three different saturated 16-carbon-long phospholipids. With an average size between only 1–2 μm they are smaller than most HS-MB (Unger *et al.,* 2004). Similar to HS-MB, lipid-based MBs have been found to be useful for therapeutic applications. They have also been successfully tested for thrombolysis in combination with US and thrombolytic agents (Unger *et al.,* 2004). Apart from Definity®/Luminity®, another SS-MB has clinical approval for cardiologic applications. SonoVue® (Bracco Imaging) gained FDA-approval in 2001 but was withdrawn, and again in 2014, came under the name Lumason®**.** This sulfur hexafluoride filled phospholipid-MB are generally used for left ventricular opafication and endocardial border definition, but in some countries also have approval for general vessel diagnostic or imaging of microvascular structures in the breast or differentiation of lesions in the liver (*Claudon et al.,* 2013; Appis *et al.,* 2015). Apart from that, SonoVue® has also been tested in clinical trials for monitoring of uterine fibroid vascularization and improved ablation (Henri *et al.,* 2014; Jiang *et al.,* 2014).

Surfactant-stabilized microbubbles

After the mid-90s usage of surfactant-stabilized gaseous MB for US diagnostics was suggested by Hilmann *et al.,* (1985).

Nanobubbles

In certain conditions, MBs, due to their size are unable to leave the vasculature, even in solid tumors, which often have leaky vasculature and a poor lymphatic drainage. This leads to extravasation and retention of macromolecules, also known as the EPR effect (enhanced permeability and retention). To extravasate to the tumor itself, bubbles need to be smaller than 400–800 nm in diameter, and are referred to as NBs. It has been shown that even bubbles of this dimension were able to produce an enhanced backscatter after US application (Oeffinger and Wheatley, 2004).

Table.1 Ultrasound contrast agent that had been clinically approved

Name	First approved for clinical use	Shell material	Gas	Application (examples)	Producer/distributor	Countries
Optison	1998	Cross-linked serum albumin	Octafluoropropane	Left ventricular opafication	GE healthcare, Buckinghamshire, UK	US, Europe
Sonazoid	2007	Phospholipid	Perfluorobutane	Myocardial perfusion, liver imaging	GE healthcare, Buckinghamshire, UK/ Daiichi Saniko, Tokyo, JP	Japan, South Korea
Lumason/SonoVue	2001/2014	Phospholipid	Sulphurhexafluoride	Left ventricular opafication, microvascular enhancement (liver and breast lesion detection)	Bracco diagnostics, Milano, Italy	US, Europe, China
Definity/Luminity	2001/2006	Phospholipid	Octafluoropropane	Echocardiography, liver/kidney imaging (Canada)	Lantheus medical Imaging, North Billerica, MA	North America, Europe (approval filed)
Imagent/Imavist	2002, withdrawn	Phospholipid	Perfluorohexane, Nitrogen	Echocardiography, heart perfusion, tumor/blood flow anomalies	Schering AG, Berlin, DE	US
Echovist	1991, withdrawn	Galactose microparticles	Air	Right heart imaging	Schering AG, Berlin, DE	Germany, UK
Levovist	1995, withdrawn	Galactose microparticles, palmitic acid	Air	Whole heart imaging, doppler imaging	Schering AG, Berlin, DE	Canada, Europe, China, Japan
Albunex	1993, withdrawn	Sonicated serum albumin	air	Transpulmonary imaging	Molecular Biosystems Inc., San Diego, CA, USA	Japan, US

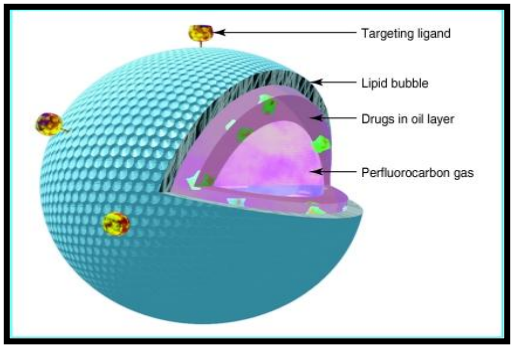
Table.2 Microbubbles and their target receptors

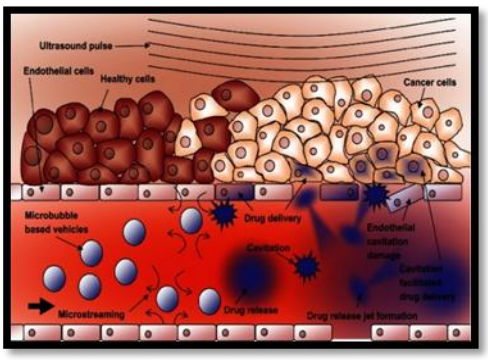
Microbubbles	Target tissue	Receptor	Ligand	Outcome
Perfluorobutane, lipid derived MB	Coronary artery endothelial cells	ICAM-1	Monoclonal antibody to ICAM-1	Significantly increased binding of MB with anti-ICAM-1
Lipid-coated perfluorocarbon containing MB	Thrombus	GPIIbIIIa-receptor	Bioconjugate ligands	Higher affinity of targeted MB versus untargeted MB
Lipid MB containing decafluorobutane	Inflamed tissue	Complement-mediated attachment to leucocytes	PS	Greater attachment of PS containing bubbles
Perfluorobutane containing MB	Avidin coated culture plates	Avidin	Biotin	Increased attachment in contrast to control MB

Table.3 Adverse reactions of contrast agent

Adverse event	Frequency 0.5-5 %	Frequency <1%
Systemic	Headache, Hypersensitivity	Abdominal pain, weakness, chest pain
Cardiovascular	Hypertension	Arterial fibrillation, palpitation and tachycardia
Digestive System	Nausea	Anorexia, diarrhoea, dyspepsia
CNS	Dizziness, Dry mouth, Vasodialation	Leg cramps, paresthesia
Respiratory, Skin		Dyspnoea, Sweating, rash and pruritis
Special Senses	Altered taste and smell	

Fig.1 Microbubble constructed for drug delivery. A stabilising material, here a lipid, surrounds the perfluorocarbon bubble. Drugs may be incorporated by themselves or, if insoluble in water, in an oil layer (Martin J K Blomley). The microsphere may be targeted to specific tissue by incorporating protein ligands on the surface.





Application of CEUS

Diagnostic imaging, medical application, organ edge delineation, echocardiography, blood volume perfusion and lesion characterization are certain applications of CEUS and are as follows.

Sentinel lymph node (SLN) detection

Lymphosonography, or CEUS-guided SLN detection, as a technique for demonstrating lymphatic drainage, has been introduced in some experimental studies. In this procedure, transcutaneous injection of an UCA is performed and CEUS is used to identify draining lymphatic channels and SLNs. This use of CEUS is technically feasible, as was demonstrated by various studies (Curry *et al.*, 2009, Wang *et al.*, 2009).

Drug or gene delivery

The microbubble contrast agent interacts with the acoustic wave in the acoustic field. The contrast agent itself will serve as a cavitation nucleus and lower the threshold level for cavitation (Wang *et al.*, 2008, Nie, *et al.*, 2006). In an experimental study, Nie *et al.*, (2008) found that compared with the group treated by US alone, KDR-tk gene therapy treated by US combined with SonoVue inhibited tumour growth and increased survival time of Hepa1-6 tumour-bearing mice. It was concluded that gene therapy mediated by US exposure enhanced by a microbubble contrast agent may become a new treatment option for HCC.

Based on the same principle, UCA was used for drug delivery in the treatment of acute intravascular thrombi by Wang, *et al.*, (2010) and Xie, *et al.*, (2009).

Molecular imaging

Techniques for non invasive imaging of specific disease-related molecular changes are being developed to enhance diagnosis and therapeutic decision-making. Molecular imaging with CEUS relies on the detection of the acoustic signal produced by microbubble or nanoparticle agents that are targeted to the sites of disease. The potential use of CEUS-based molecular imaging in atherosclerosis, post-ischemic inflammation, angiogenesis, transplant rejection and thrombus formation have been investigated, and is undoubtedly an important development trend (Linder, 2009, Kaufmann, *et al.*, 2007, Bohman, *et al.*, 2009, Leong Poi, *et al.*, 2007 and Lankford, *et al.*, 2006).

Commercially available contrast agents

There are different types of contrast agents which are available in the market like simple air (Nitrogen), perfluorocarbon and sulfur hexachloride preparations (Vera Paefgen *et al.*, 2015) Table.1.

Procedure for CEUS

After preparation the target organ is focused on B-mode US and then contrast-specific imaging mode is turned on. On ultrasound after the contrast is administered, the tissue is divided on basis of perfussion i.e. hyperenhancing, isoenhancing, hypoenhancing.

Limitations of technique

Different limitations regarding contrast enhanced ultrasound are ultrasound produces more heat as the frequency increases, Microbubbles don't last very long in circulation, Continues monitoring is required, Microbubbles burst at low ultrasound frequencies and may cause local microvasculature rupture and haemolysis and Costly with time consuming. Contrast enhanced ultrasound helps in different aspect likewise in absolute quantification of tissue perfusion, Helps in drugs and genes delivery, Lower intravenous dosage is needed, Guided organ transplantation, Localization of lesion, Helpful for the differential diagnosis, Differentiation between malignant & benign tumour and Monitoring response of tumor therapy.

Abbreviation

CEUS - Contrast enhanced ultrasound
MB - Microbubble
NB - Nanobubble
USCA - Ultrasound contrast agent
BBB - Blood brain barrier

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