Small Ruminant Research 152 (2017) 33–40

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Contrast-enhanced ultrasound in sheep

K. Vanderperren[a,*], E. Stock[a], B. Pardon[b], J. Saunders[a]

[a] Department of Veterinary Medical Imaging and Small Animal Orthopedics, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[b] Department of Internal medicine and clinical biology of large animals, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

ARTICLE INFO

Article history:
Available online 19 December 2016

Keywords:
Abdominal organs
Contrast-enhanced ultrasound
Microbubble
Sheep

ABSTRACT

Contrast-enhanced ultrasound (CEUS) is a recent imaging technique that uses microbubble contrast agents to obtain additional information relating to tissue vascularity and tissue perfusion. In veterinary medicine, CEUS has proven its usefulness in a large variety of diseases, mainly in dogs and cats. The most common indication is differentiation between benign and malignant lesions in abdominal organs, frequently in the liver. Although only a few experimental reports in sheep are available in literature, CEUS is of particular interest in ovine diseases involving disturbances in vascularisation, e.g., vascular, inflammatory or neoplastic disorders. The article reviews the basic physics of CEUS and different contrast media. Also, some practical considerations about the procedure are mentioned. Current and future potential clinical applications in sheep are discussed. Safety matters and contraindications are also described.

1. Introduction

Ultrasonographic examination is a valuable tool for medical diagnosis, offering non-invasive, real-time imaging with excellent spatial resolution. Contrast-enhanced ultrasound (CEUS) is a recent imaging technique that uses microbubble contrast agents to obtain additional information relating to tissue vascularity and tissue perfusion. In humans, CEUS has been used in a wide range of disorders of the abdominal organs (Forsberg et al., 1999; Liu et al., 2008), myocardium (Vogel et al., 2005), even the brain (Rim et al., 2001). In veterinary medicine, several reports on the application of CEUS have been published, mostly in dogs and cats, where CEUS has proven its usefulness in a large variety of diseases, particularly in differentiation between benign and malignant lesions.

The technique has been used in sheep, specifically in experimental model studies for human applications. To our knowledge, no clinical reports have been published about the use of CEUS in that species. As ultrasound equipment is widely available in general veterinary practice and given that, occasionally (e.g., pet sheep, expensive animals of high reproductive value), owners may be willing to pay for a more expensive diagnostic work-up, use of CEUS could become more widespread. It is noteworthy that no maximum residue levels for specific use of microbubbles in sheep have been set; hence, their use in those animals should be followed by prescribing a withdrawal period that needs to be set by the prescribing veterinarian and should be >7 days for milk and >28 days for meat.

Objective of this review article is to provide a comprehensive overview of basic principles of CEUS, the different discussed media and the safety of CEUS. Furthermore, practical considerations about the procedure are mentioned. Finally, current and future potential applications in sheep in research model for humans or in potential clinical applications in that species are discussed.

2. Contrast-enhanced ultrasound procedure

2.1. Basic principles of contrast-enhanced ultrasound

Ultrasonographic examination is one of the most widespread imaging methods used in medical practice. Its accessibility, low cost, real-time and non-invasive characters are all factors that contribute to the popularity of the method. Ultrasonographic examination is a non-invasive imaging tool for assessment of size, shape, parenchymal texture and vascularity of various organs (Haers and Saunders, 2009). Both conventional colour and spectral Doppler, nowadays available on a wide series of ultrasound devices, have strong limitations for imaging of perfusion (Wilson et al., 2009). Although Doppler imaging may provide valuable directional blood flow information, it is only effective for evaluation of large blood vessels with fast-flowing blood and cannot detect flow of a low volume or velocity or flow from unfavourable angles or in

* Corresponding author.
E-mail address: katrien.vanderperren@ugent.be (K. Vanderperren).

http://dx.doi.org/10.1016/j.smallrumres.2016.12.017
0921-4488/

deep tissues (Rubin et al., 1994; Nilsson et al., 1997; Wei et al., 2001; Correas et al., 2006). Further, Doppler is very sensitive to motion artefacts, making it sometimes difficult to use in awake or panting animals.

Contrast-enhanced ultrasonography (CEUS) is a recent real-time imaging modality that provides a unique means of visualising tissue perfusion at capillary level. The scattering ability of the blood can be enhanced with gas-containing microbubbles, termed 'ultrasound contrast agents' (USCAs). Ultrasound contrast agents rely on the different ways in which sound waves are reflected from interfaces between substances (Haers and Saunders, 2009). This may be the surface of a small air bubble or a more complex structure. Gas-containing microbubbles, which are mainly administrated intravenously in the systemic circulation, have a high degree of echogenicity, because acoustic difference between gas into the microbubbles and soft tissues surroundings of the body is immense. Thus, ultrasonic imaging using USCAs enhances ultrasound backscatter, or reflection of the ultrasound waves, to produce a unique sonogram with increased contrast due to high echogenicity difference. Therefore, even tissue parenchymal microcirculation can be detected.

Contrast-specific imaging technology exploiting the specific acoustic properties of USCAs enables continuous real-time imaging of tissue parenchymal blood flow, by suppressing signals from tissues, thus, intensifying signals from USCAs (de Jong et al., 2001; Greis, 2004). Several techniques have been developed for signal processing to selectively collect information from non-linear microbubble oscillations; these include second harmonic imaging (Schrope and Newhouse, 1993), pulse inversion harmonic imaging (Simpson et al., 1999) and cadence-contrast pulse sequencing and power (amplitude) modulation (de Jong et al., 2001; Thomas et al., 2009).

Contrast-specific detection modes utilise the interaction of the microbubbles with ultrasound waves. This interaction depends on microbubble size, shell flexibility, transducer frequency and mechanical index (MI) (Church, 1995; de Jong et al., 2000; Uhlendorf et al., 2000; Emmer et al., 2007; Greis, 2004). The mechanical index varies, depending on the ultrasound machine, but is basically conditioned by the acoustic power of the ultrasounds beam. The acoustic power, measured in pascals (Pa), represents the energy of the sound beam acting on a target, e.g., a group of red cells or the contrast agent inside the blood stream. At very low acoustic power, oscillation of microbubbles is symmetrical and the change in size is equal in both compression and expansion (Hoff, 1996; Correas et al., 2001; Kollmann, 2007). At low values of the acoustic power (30–70 kPa), microbubbles start to expand more than compress, and, thus, vibrate in a particular, non-linear manner, producing alternating contractions and relaxations, that way generating harmonic echoes (Hoff, 1996). However, at high acoustic power (up to the order of megapascals), microbubbles are 'broken' and an irregular, non-linear signal is generated (Correas et al., 2001; Kollmann, 2007).

The goal of contrast-specific imaging techniques is to separate the signal from the microbubbles from the signal caused by the surrounding tissue. Earlier techniques were based on filtering the harmonic signals; the fundamental frequency was blocked and only the harmonics were used to construct the image; complete separation of the signals from tissue and microbubbles could be obtained by this technique. Another approach is to transmit two pulses, with the second being reversed in phase from the first one; this technique is called 'pulse inversion'. Echoes from both pulses are summed to form the image; signals returning from tissues are opposite and cancel each other and are thus not displayed. Signals from non-linearly behaving microbubbles are summed (Correas et al., 2001; Kollmann, 2007; Haers and Saunders, 2009). Nowadays, 'pulse-cancellation imaging' or 'Cadence Contrast Pulse Sequence' techniques are used. Multiple pulses of varying phase and amplitude are produced; these pulses are then processed to separate the signals from tissue and microbubbles (Kollmann, 2007; Haers and Saunders, 2009).

2.2. Contrast agents

Ultrasound contrast agent (USCA) consists of very small gas-filled microbubbles that are encapsulated by a shell. The microbubbles are 1–7 μm in diameter, which are close in size to red blood cells. Due to their extremely small size, microbubbles pass through the pulmonary circulation without causing embolism and then disseminate into the systemic circulation through the arterial blood stream. Furthermore, they cannot pass the vascular endothelium and thus remain strictly intravascular (Correas et al., 2001; Tang et al., 2011). Recently developed microbubbles contain an inert, high molecular weight gas dissolving slowly in the blood (Haers and Saunders 2009). The contrast agent remains in the bloodstream for about 4–5 min. Some USCAs (Sonazoid, Sonavist, Levovist) have a hepato-/spleno-specific affinity in the late parenchymal phase that is probably due to pooling of the microbubbles in the hepatic sinusoids or due to phagocytosis by the Kuppfer cells (Forsberg et al., 1999). The gas content of the microspheres is typically eliminated from the lungs by exhalation after 10–15 min, whereas shell components are filtered by the kidneys and eliminated by the liver (Correas et al., 2001; Haers and Saunders 2009).

There are a variety of commercially available microbubble contrast agents, which differ in their shell and gas core makeup. The selection of the shell material determines how easily the microbubbles are taken up by the immune system. A more hydrophilic material tends to be taken up more easily, which reduces the microbubble residence time in circulation, therefore the imaging time. Secondly, the shell material affects also the microbubble mechanical elasticity: the more elastic the shell, the more acoustic energy it can withstand before bursting. Currently, microbubble shells are composed of albumin, galactose, lipid or polymers (Correas et al., 2001).

The gas core of the microbubbles is the most important part, because this determines the echogenicity. Microbubbles will compress, oscillate and reflect a characteristic echoes under influence of an ultrasonic frequency field. Gas cores can be composed of air or heavy gases, e.g., perfluorocarbon or nitrogen. However, inert gases (perfluor gases) with higher molecular weight provide more advantages compared to air, because they dissolve more slowly in the blood, meaning a longer half-life, resulting in a longer lasting signal enhancement (Haers and Saunders 2009). However, once the USCAs are reconstituted, they have a short-term efficacy (6–12 h).

The commercial development of USCAs started in the 80s. The most commonly used USCAs in veterinary medicine are perflutren lipid microspheres (Definity; Lantheus, N. Billerica, USA) and phospholipid-stabilised sulfur hexafluoride microspheres (SonoVue; Bracco, Milan, Italy) (Seiler et al., 2013). The various contrast agents are divided into first or second generations microbubbles, depending on the gas contained; first generation products contain air, whereas second generation ones are filled with high-molecular weight gases.

2.3. Safety, adverse reaction and contraindications of contrast agents

In general, USCAs are well-tolerated for abdominal examinations, with few adverse reactions reported, whereas their use is contraindicated in patients with unstable cardiopulmonary status (Appis et al., 2015). Moreover, in contrast to iodinated or gadolium-based contrast agents, USCAs are not nephrotoxic and can be used safely in patients with renal impairment (Dong et al., 2013). In