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● *Original Contribution*

EFFECT OF HIGH-INTENSITY ULTRASOUND–TARGETED MICROBUBBLE DESTRUCTION ON PERFUSION AND FUNCTION OF THE RAT HEART ASSESSED BY PINHOLE-GATED SPECT

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Abstract—Although ultrasound-targeted microbubble destruction (UTMD) has been shown to induce bioeffects, UTMD is still desirable for therapeutic applications. Therefore, we studied the effects of UTMD on perfusion and function of the rat heart, assessed by ^{99m}Tc-MIBI pinhole-gated SPECT (Ph-gSPECT) compared with biomarker release and histopathology. Fifty-two male Wistar rats were studied. UTMD was performed using SonoVue, with a mechanical index of 1.0 or 1.6. Controls were treated without microbubbles or without ultrasound application. At baseline, day 1, day 7 and day 30, 35 rats were imaged with ^{99m}Tc-MIBI Ph-gSPECT to quantify left ventricular perfusion and function. In addition, troponin release and histopathology were investigated. No significant differences were observed for left ventricular ejection fractions, end-systolic and end-diastolic volumes, regional perfusion and functional scores up to 30 days after UTMD compared with controls. UTMD induced mild troponin release and early erythrocyte extravasation without necrosis, inflammation or fibrosis. Although UTMD has the potential to induce microlesions of the heart in small animals, these effects were transient without histological evidence of irreversible damage. Furthermore, UTMD does not induce abnormalities on perfusion or function of the heart, as assessed by Ph-gSPECT, which is reassuring concerning the use of SonoVue for potential therapeutic applications. (E-mail: sophie.hernot@gmail.com) © **2010 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Microbubble, Ultrasound-targeted microbubble destruction, Bioeffects, Pinhole-gated SPECT, Troponin.**

INTRODUCTION

Ultrasound contrast agents are now frequently used in the clinical area of echocardiography and radiology (Aggeli et al. 2008; Albrecht et al. 2004; Kusnetzky et al. 2008; Lindner and Wei 2002). Since 2001, more than 2 million vials of approved contrast agents have been used in patients in the United States (Main et al. 2007). Moreover, because of promising progress concerning targeted microbubbles (Klibanov 2007), their application field will continue to expand.

However, it has been shown that the destruction of microbubbles with high-intensity ultrasound can cause bio-effects in small animals (Miller 2007) and induce mild troponin release and premature ventricular contractions in humans (van der Wouw et al. 2000; Vancraeynest et al. 2007). To some extent, these bio-effects may open opportunities for therapeutic applications (Hernot and Klibanov 2008). The overlap between therapeutic and toxic effects of ultrasound-targeted microbubble destruction (UTMD) remains unclear, especially regarding the potential of reversibility of these bio-effects. Although the capability of high-intensity UTMD to induce histologically definable microlesions in small animals has been demonstrated (Miller et al. 2005b), the effects of such microlesions on both perfusion and function of the heart have not yet been examined.

Gated SPECT is an established technique for the assessment of coronary artery disease in humans and has been validated recently for normal and diseased small animals (Cosyns et al. 2007; Maskali et al. 2006; Vanhove et al. 2005) by the use of pinhole collimators and advances

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in image reconstruction (Vanhove et al. 2000). Pinhole-gated SPECT (Ph-gSPECT) with technetium-99m methoxyisobutylisonitrile (^{99m}Tc-MIBI) as perfusion tracer offers the unique opportunity to evaluate regional myocardial perfusion along with global and regional left ventricular function with a high sensitivity.

Therefore, we sought to determine the impact of microbubble destruction induced by high-intensity ultrasound on both perfusion and function of the heart in rats, studied with ^{99m}Tc-MIBI Ph-gSPECT, compared with early biomarker release and histological findings.

MATERIALS AND METHODS

Experimental setup and animal handling

Fifty-two male Wistar rats (Harlan, Horst, The Netherlands) 10 –12 weeks old (weight range 347 ± 19 g) were studied. Among these, 35 rats were divided into five experimental groups (Fig. 1). Group 1 (n=10) and group 2 (n=10) underwent UTMD with a mechanical index (MI) of 1.0 and 1.6, respectively. The three other groups were considered control groups and treated either with microbubbles only (n=5) or with ultrasound only (n=5 for MI 1.0 and n=5 for MI 1.6) (in respective order group 3, 4 and 5). The rats were assigned to the different groups in random order.

UTMD or control treatment was performed at day 0 as described next. All animals were imaged with ^{99m}Tc-MIBI Ph-gSPECT 5 days before UTMD application (baseline), and at day 1, 7 and 30. Blood samples were also taken at day 1 for the troponin assay. At the end of the experiment at day 30, the animals were killed by intravenous injection of 70 mg/kg sodium pentobarbital (CEVA, Brussels, Belgium). The hearts were harvested immediately for further histological examination. Additional rats were used to immediately evaluate the microscopic lesions (n=6 for MI 1.0, n=5 for MI 1.6) and at day 1 (n=3 for MI 1.0, n=3 for MI 1.6) after UTMD. In a subset of five rats (n=3 for MI 1.0, n=2 for MI 1.6), Evans blue dye (50 mg/kg) was injected before UTMD to demonstrate microvascular leakage (Fig. 1).

During the entire study, the animals were housed in stainless steel cages with sawdust bedding. They were kept at an average room temperature of 24°C, a relative humidity of 50% and a 12- hour day/night cycle. All rats had unlimited access to food and water during follow-up. The study protocol was approved by the Ethics Committee for animal studies of the Vrije Universiteit Brussel. Guidelines of the National Institute of Health Principles of Laboratory Animal Care were followed.

Ultrasound-targeted microbubble destruction

The rats were anesthetized using intraperitoneal injection of pentobarbital (50 mg/kg) 15 min before the experiment, and the thoracic region was shaved. The rats were placed in supine position. A 21-gauge cannula was inserted into the tail vein and SonoVue (Bracco SPA, Milan, Italy) (lipid-coated microbubbles filled with sulfur hexafluoride; 2.5×10^8 bubbles/mL) was infused during ultrasound exposure at a constant rate of 0.3 mL/min using a dedicated pump (Bracco Research). Electrodes were attached to the paws for electrocardiogram (ECG) triggering. The cardiac phased-array scanhead 3S or S3 of a diagnostic ultrasound device (respectively, Vivid 7 Pro, GE VingMed, Horton, Norway, and Sonos 5500, Philips Medical Systems, Andover, MA, USA) was clamped

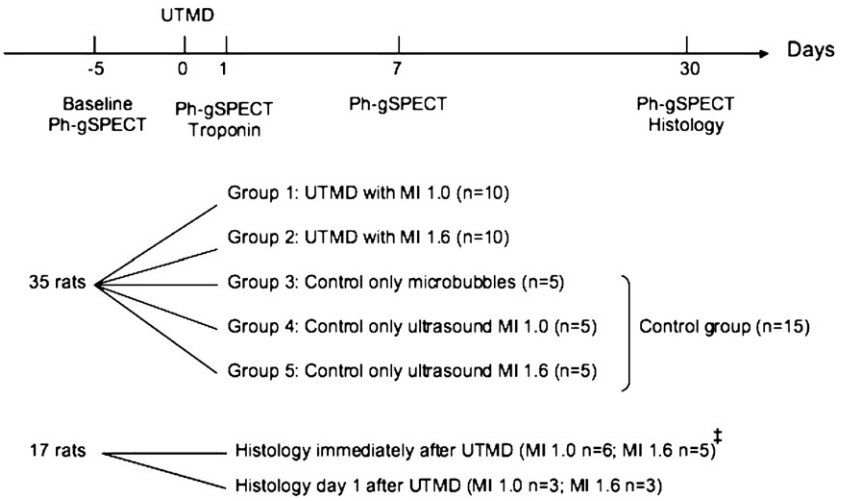

Fig. 1. Schematic representation of the experimental protocol. Thirty-five animals underwent UTMD or control treatment at day 0. At baseline, day 1, day 7 and day 30 after UTMD or control treatment, the animals were subjected to ^{99m}Tc-MIBI Ph-gSPECT. At day 1, blood samples were taken for troponin analysis. Additional rats were used for histologic evaluation immediately and at day 1 after UTMD. ‡In a subset of five of these rats, Evans blue was injected before UTMD.