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Acoustic characterization of a new trisacryl contrast agent. Part II: Flow phantom study and in vivo quantification

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Abstract

The biocompatible trisacryl particles (TMP) are made of a cross-linked acrylic copolymer. Their inherent acoustic properties, studied for a contrast agent application, have been previously demonstrated in a in vitro Couette device. To measure their acoustic behaviour under circulating blood conditions, the TMP backscatter enhancement was further evaluated on a home-made flow phantom at different TMP doses (0.12–15.6 mg/ml) suspended in aqueous and blood media, and in nude mice (aorta and B16 grafted melanoma). Integrated backscatter (IB) was measured by spectral analysis of the Doppler signals recorded from an ultrasound system (Aplio®) combined with a 12-MHz probe. Doppler phantom experiments revealed a maximal IB of 17 ± 0.88 dB and 7.5 ± 0.7 dB in aqueous and blood media, respectively. IB measured on mice aorta, in pulsed Doppler mode, confirmed a constant maximal value of 7.29 ± 1.72 dB over the first minutes after injection of a 7.8 mg/ml TMP suspension. Following the injection, a 60% enhancement of intratumoral vascularization detection was observed in power Doppler mode. A preliminary histological study revealed inert presence of some TMP in lungs 8 and 16 days after injection.

Doppler phantom experiments on whole blood allowed to anticipate the in vivo acoustic behaviour. Both protocols demonstrated TMP effectiveness in significantly increasing Doppler signal intensity and intratumoral vascularization detection. However, it was also shown that blood conditions seemed to shadow the TMP contrast effect, as compared to in vitro observations. These results encourage further investigations on the specific TMP targeting and on their bio-distribution in the different tissues.

Keywords: Ultrasound; Contrast agent; Polymeric microparticle; Trisacryl; In vivo; B16 melanoma; Doppler spectrum; Integrated backscattering

1. Introduction

In malignant tumors, vascularization by angiogenesis correlates with invasive potential and among existing modalities, ultrasound (US) has provided promising evaluation of this tumoral vascular network [1]. Indeed, with Doppler US, blood flow is detectable in larger vessels and can be quantified using computerized tools. However, the access to smaller vessels such as capillaries for the perfusion measurement, is a difficult task [2] considering the slow velocity and irregular nature of vessels, particularly in tumor vasculature, and conventional power Doppler US is capable of visualizing capillary blood flow in vessels above 80 μm [3]. The sensitivity of diagnostic US imaging can then be improved by intravenous injection of vascular contrast agents, which are known to significantly enhance the acoustic backscattering from blood in both color and spectral Doppler ultrasound modes [4,5]. These contrast agents have involved many recent

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US applications for assessing the neovascularity of tumors, following the effects of therapy or also predicting the potential metastatic evolution from a primary tumor [6]. Trisacryl particles have proved to be effective, biocompatible and safe in the embolization application of clinical studies [7] and have been approved by the Food and Drug Administration as successful emboles for treatment of hypervascular tumors [8]. Moreover, these embolic agents of 200–1000 μm have recently demonstrated some inherent ultrasound properties in vitro and in vivo [9] and smaller derived particles with diameter around 2 μm, have still revealed interesting imaging properties in vitro (Lavisse et al. Part I). Actually, in the first part of the study, trisacryl microparticles (TMP) have been characterized in vitro through backscatter and attenuation parameters according to concentration, emission frequency in the range of 3–17 MHz, and acoustic pressure. In that first study, the TMP backscatter showed enhancement up to 16 dB for the most concentrated suspensions (7.8 and 15.6 mg/ml). However, each parameter measured under the different conditions, was deduced from TMP suspended in a reference medium of physiological serum and glycerol and was analysed on a specific Couette flow set-up. Further investigations need now to be conducted to approach in vivo conditions in terms of flow and blood environment. Indeed, we hypothesize that there might be differences between the in vitro acoustic behaviour of TMP under US exposure in an aqueous medium as compared with acoustic behaviour in blood, resulting from possible density, viscosity and influence of many additional molecular species present in blood [10]. The objective of this study was hence to further investigate the ultrasound properties of the trisacryl microparticles suspended in the blood and according to two different protocols. The first one was performed using a home-made flow phantom usually designed for US studies and assessment of Doppler flow measurements at flow velocities comparable to those in blood vessels of the macrocirculation [11,12]. The second protocol was conducted in vivo after intravenous injection of trisacryl microparticles. For both protocols, backscattering properties were evaluated by the native detected Doppler signals. Indeed, as the spectral density of the Doppler signals corresponds to the energy distribution of all velocities contained in the measured volume, it has already been well demonstrated [13,14] that Doppler signals contain not only flow velocity information, but can also be used for quantifying (by spectral density integration) ultrasonic backscatter on flow systems.

In addition, to study the biocompatibility of the TMP after intravenous injection, we performed in this part histological examinations of different organs removed after in vivo experiments.

For all experiments, the ultrasound field parameters are reported according to the "Guidelines for *Journal of Ultrasound in Medicine* Authors and Reviewers on Measurement and Reporting of Acoustic Output and Exposure" [15].

2. In vitro quantification

2.1. Experimental flow phantom

To investigate the contrast enhancement induced by the TMP in a flow model, particles were imaged in an experimental set-up composed of a flow phantom connected to a sonograph (Aplio®, Toshiba Medical, Puteaux, France) and a digital scope (Wavepro® 950, Lecroy, Courtaboeuf, France). The flow phantom consisted of a continuous flow-roller pump (pump drive PD 5101, Heidolph Instruments, Schwabach, Germany) pumping suspensions at variable rates between 1.5 and 10 cm/s through a tygon tube with inner diameter of 2.4 mm (unloaded). This mimicking vessel was 25 cm in length and was embedded into a plastic cubic tank (28.5 cm length, 12 cm width, 10.5 cm height) filled with degassed water at room temperature, inserted just before experiments (Fig. 1). The tank bottom was recoverd with 10-mm-thick-ultrasound absorber walls (Aptflex- NPL® F28, Precision Acoustics Ltd., Dorchester, UK) in order to reduce acoustic reflections. The exposure volume inside the mimicking vessel was of 2.6 ml and the total volume of the circulating suspension inside the entire system was 10 ml including the 0.5 ml funnel reservoir. This reservoir prevented microparticle sedimentation and was also used to maintain a constant solution concentration in the tube. The flow pump was calibrated by collecting the volume of liquid pumped over a fixed interval of time: for flow velocity set at 4.8 cm/s, the continuous flow through the model was achieved at 12 ml/min. A 12-MHz linear probe (PLT1024, Toshiba Medical, Puteaux, France) associated to an ultrasound Aplio® system was immersed in the water tank and fixed 3 cm above the upper part of the tube, at an angle of 25° with regard to the particles flow. The linear probe contained 192 elements, designed according to the shape of a rectangular aperture (34 mm × 7 mm, height and width), and worked with geometric (13 mm focal length) and electronic focussing allowing a focus depth range of 2.5–470 mm. The acoustic power

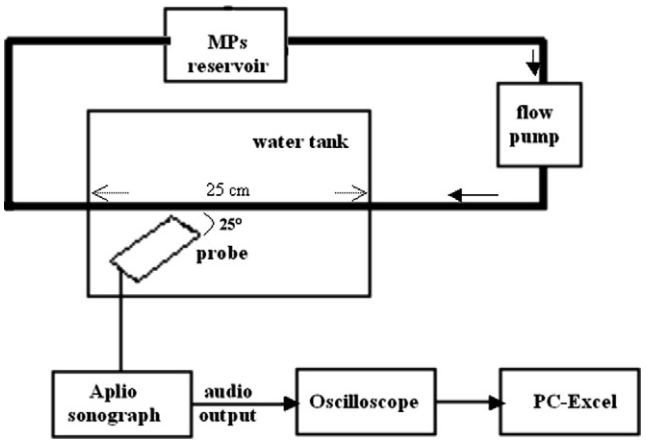

Fig. 1. Experimental set-up of the flow model composed of a flow phantom connected to a sonograph Aplio® and a digital scope.