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ScienceDirect
Ultrasonics xxx (2008) xxx–xxx
Ultrasonics
www.elsevier.com/locate/ultras

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

Sonia Lavisse [a], Pierre Peronneau [a], Valerie Rouffiac [a], Angelo Paci [b], Julie Vigouroux [a], Paule Opolon [c], Alain Roche [a], Nathalie Lassau [a,*]

[a] *Universite de Paris-Sud, Imaging Department and UPRES EA 4040, Orsay F-91405, Institut Gustave Roussy, 39, Rue Camille Desmoulins, 94805 Villejuif Cedex, France*
[b] *Département de Pharmacie Clinique, Institut Gustave Roussy, Villejuif, France*
[c] *UMR8121 Vectorisation et transfert de gènes, Institut Gustave Roussy, Villejuif, France*

Received 11 July 2007; received in revised form 10 September 2007; accepted 10 October 2007

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

* Corresponding author. Tel.: +33 1 42 11 60 14; fax: +33 1 42 11 60 29.
E-mail address: nathalie.lassau@igr.fr (N. Lassau).

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doi:10.1016/j.ultras.2007.10.006

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

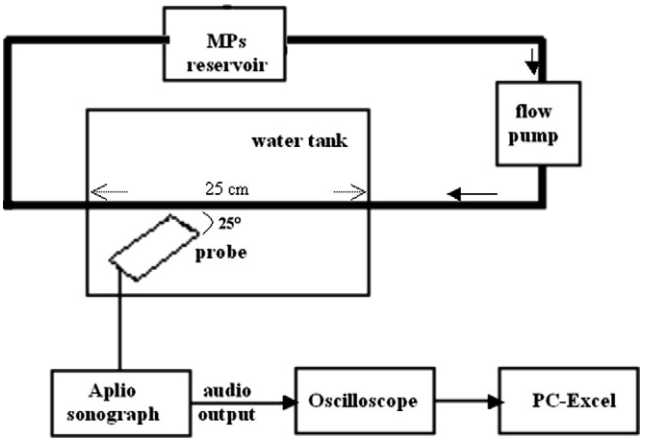

Fig. 1. Experimental set-up of the flow model composed of a flow phantom connected to a sonograph Aplio® and a digital scope.

at transducer was of 19.9 mW. The probe was coupled to the phantom so that the tube, when filled with water, appeared in the image as a narrow parallelogram in the focus of the scan plane. This probe allowed to image the particle flow in fundamental B and pulsed Doppler modes. The B and pulsed Doppler modes emitted respectively 250 ns and 750 ns duration pulses with a 14 MHz and 8.9 MHz center frequency, at a 1 kHz repetition frequency (PRF) for both. The ultrasound focal zone was fixed at the middle of the tube visualized in B-mode (frame rate of 12 Hz). The constant volume of Doppler signal measurement was positioned in order to cover only the whole section of the tube for each particle concentration. In pulsed Doppler mode, the 12-MHz probe was calibrated in an ultrasound test tank containing an hydrophone (HGL 0200- PZT-Onda Corporation®, Sunnyvale, CA, USA) (Lavisse et al. Part I) and emission frequency of this probe was measured at 9.35 MHz.

2.2. MP suspension

Trisacryl microparticles were provided by Biosphere Medical (Roissy-en-France, France). Microparticles were suspended in two different media: the first medium was composed of glycerol and physiological serum (40:60, v/v) to simulate blood viscosity (4 cP) and the second one consisted of whole human blood (provided by the Etablissement Français du Sang, Rungis, France). TMP were injected into the funnel at six different dosages between 0.12 mg/ml (2.33×10^7 particles/ml) and 15.62 mg/ml (3.63×10^9 particles/ml). Four experiments were led for each concentration and medium. A complete replacement of the suspension was performed between each concentration experiment to avoid cumulative contrast effect.

Doppler data were recorded first with medium alone and then with suspended trisacryl particles after a stabilization period.

To calibrate the phantom set-up, preliminary measurements were performed using the 12 MHz linear probe with increasing concentrations of talcum powder. At low concentrations, the integrated backscatter (IB) is indeed increasing linearly with the dose.

2.3. Acquisition system and characterization parameters

Characterization of particle backscattering was performed by quantifying the change in Doppler intensity measured inside the tube in the reference medium and in the medium suspending the TMP. For this purpose, the native Doppler signals were extracted from the sonograph and transferred to the 950 WavePro® digital scope (8 bits resolution). These analogue signals were digitized on 8 bits with a 500 KHz sample rate and recorded for 20 s. For each measurement, five Fourier transforms were calculated using a Hamming window. The corresponding Doppler spectra were then filtered with a moving low pass filter of size 5 on an Excel® 9.0 software (Microsoft Corporation, Courtaboeuf, France). Integrated backscattering was extracted from 50 to 600 Hz (wall filter = 61 Hz, Doppler frequency ≈ 300 Hz) and was seen as the measure of the total ultrasonic energy backscattered by the interrogated volume of the tube. Each integrated backscattering value was then normalized by subtracting the mean pre-contrast Doppler spectrum integration from the one after particle injection. After normalization, integrated backscattering of all experiments was averaged and referred as IB.

3. In vivo investigations

3.1. Backscatter quantification studies and tumoral model

Echo-Doppler experiments were performed on the Aplio® ultrasound scanner equipped with the same 12-MHz probe. Particle enhancement was quantified according to two different studies: (1) a kinetic study was performed on the aorta of 10 mice and was based on the native Doppler signals recorded by the 12-MHz-probe before and every minute over 10 min after particle injection; (2) a quantitative study of detectable intratumoral vessels was realized before and after particle injection into 13 melanoma-grafted mice to calculate variation of intra-tumoral vascularization detection in power Doppler mode (8.8 MHz pulse center frequency and 750-ns pulse duration).

Each mouse was imaged through a 0.4-cm ultrasound gel standoff heated to body temperature.

For the second protocol, B16F10 melanoma cells (CRL-6475, ATCC, LGC PromoChem, Molsheim, France) were prepared and cultured in DMEM medium, 10% fetal bovine serum, penicillin/streptomycin and glutamate (Invitrogen Life Technologies Inc., Cergy Pontoise, France). Two millions of melanoma cells were subcutaneously injected in the right flank of 13 nude mice. Ultrasound investigations started with tumoral volumes ranged from 80 to 400 mm^3.

3.2. In vivo protocols

All animal experiments were conducted with locally bred mice, in accordance with the European Community guidelines (Directive no. 86/609/CEE). Mice were anesthetized with a 0.2 ml intraperitoneal injection of a solution containing ketamine hydrochloride (50 mg/ml), rompun (2%) and sterilized water. Mice were placed on an experimentation table connected to a pump with adjustable thermostat constituting a continuous hot water circulating system (Gaymar, TEM, Bordeaux, France) to prevent mice hypothermia during and after examinations. For all experiments, particles were prepared in a 156 mg/ml particle solution suspended in physiological serum. A volume of 100 μL of TMP was injected, using a 1-mL syringe connected to a 30-gauge needle (MicroLance, Becton Dickinson, Le Pont-De-Claix, France), which was inserted into the retro-orbital vein of the mouse. The 156 mg/ml

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concentration was used in order to achieve 7.8 mg/ml in situ (1.8×10^9 particles/ml) according to the 2 ml mouse blood volume.

For the kinetic study, a control group was analysed with physiological serum injection. For all animals, Doppler ultrasonic signals measured on the aorta (tissue thickness from transducer = 1 cm) were digitized and processed under the same conditions than the phantom model protocol.

In the second quantitative study, power Doppler mode with the 12-MHz probe allowed to visualize and evaluate intra-tumoral vascularization. Gain, frame rate (12 frames/s) and limit detection (4.3 cm/s) were adjusted at the initiation of the experiments to avoid colour artefact and were maintained constant throughout the experiment. This second in vivo protocol consisted of first calculating the tumoral volume in B-mode with length, width and thickness measurements on the maximal longitudinal and transversal sections.

Blood flow imaging was then performed before particle injection to record reference vascularization sequences. A new Doppler sequence was again initiated after particle injection. For each video sequence, the whole tumor volume was scanned twice along transversal and longitudinal plans by continuously displacing the 12-MHz probe across successive sections. Sonographic scans on both plans were recorded on digital videotapes and were post-reviewed by two independent observers. These observers reviewed all the cine loops and evaluated, for each examination, the intra-tumoral vessel number on the whole tumor volume in longitudinal and in transversal plans. Color pixel clots were considered as markers of an intra-tumoral vessel when these pixels were repeatedly found in successive tumor sections showing a continuous follow-up of the blood flow. For each mouse, each observer averaged the intra-tumoral vessels evaluated in the two orthogonal plans. The intra-tumoral vessel number in the whole tumoral volume was finally defined as the mean of vessels evaluated by the two operators [1].

3.3. Viscosity measurements

To test a potential change of blood viscosity after particle injection into the retro-orbital sinus, blood volume was collected from eight control nude mice receiving 0.1 ml physiological serum injection and from 9 mice used in the in vivo investigations after a 0.1 ml particle injection volume (156 mg/ml). A total of 6 ml and 7 ml blood without and with particles, respectively were collected in capillary heparin tubes and transferred into a low-shear viscosimeter (Low Shear 30, Contraves AG, Zürich, Switzerland). The available blood volumes allowed to record two measurement points for each sample without and with particles. Measurements of hematocrit rate and viscosity were performed on the same day of sacrifice and the day after (samples stored in the fridge) at different shear rates of 241, 96, 51.9, 11.2, 2.41 and 0.519 s^{-1}.

4. Preliminary toxicity study

Fifteen mice were injected at Day 0 (D0): twelve mice (GP1) were injected with 0.1 ml of the 156 mg/ml particles solution and three other mice (GP2) were injected with physiological serum for toxicity control. At D0, 4 GP1-mice and one GP2-mouse were sacrificed. At D8 and D16, four mice of GP1 and one control in GP2 were sacrificed. Mice were euthanised with CO_2 gaz according to guidelines established by the Institutional Animal Care and Use Committee. Liver, kidneys, heart, lungs and spleen were autopsied, fixed in FineFix solution (Milestone, Sorisole, Italy) and embedded in paraffin. Four micrometer sections were prepared, stained with HES and then examined by a pathologist to assess the potential toxicity of the particles.

5. Statistical study

In the kinetic study, the enhancement variations between pre- and post-TMP injection at different times was evaluated on XLStat®2006 (Addinsoft, Paris, France) by a Mann and Whitney test at significance levels of $p < 0.05$. To correlate the intratumoral vessel number with volume and pre-injection vessel number, we used a linear regression associated to a Pearson's correlation coefficient.

6. Backscatter parameter on the flow phantom

Backscatter values were measured on the phantom device when particles were suspended first in the reference medium composed of glycerol and physiological serum and then in human blood. The Fig. 2 shows IB expressed as a function of particle dose, when suspended in both media. With the glycerol mixture as reference, IB increased with concentration up to a maximal value of 17.2 ± 0.88 dB (65.2 in linear a.u.). By representing IB in linear data, backscattering intensity was found to linearly increase with the dose ($R^2 = 0.96$) up to 7.8 mg/ml with then a plateau related to a multiple scattering phenomenon. This result has to be correlated with the IB-dose relation found in the Couette device study (Lavisse et al. part I). However, when suspended in blood, particles exhibited a lower backscatter, increasing with concentration up to 7.5 ± 0.7 dB at the maximal dose. This result could be expected as blood is known to contain red cells acting like ultrasound scatterers, which can potentially shadow the TMP effect. Moreover, preliminary experiments on blood had already been conducted on the Couette device under different shear stress conditions and observations had been found equivalent to those on the flow phantom (data not shown).

Without particle, flow mean velocity of the glycerol mixture could be extracted from Doppler spectra and was found at 4.51 ± 0.12 cm/s (211.3 ± 5.6 Hz). This value was in the same order than the theoretical velocity imposed by the pump (4.8 cm/s).

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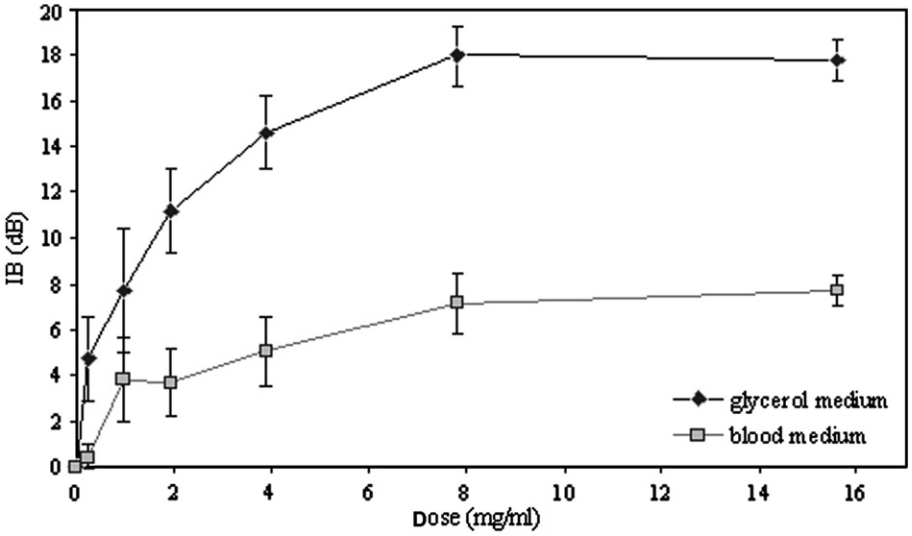

Fig. 2. Mean values of IB from trisacryl particles suspended in 40%-glycerol medium (line in black) and in human blood (line in grey). (errors bars indicating the average within the 4 experiments).

7. Viscosity measurements

The 6 and 7 ml blood volumes, collected from control nude mice and mice receiving a 0.1 ml particle injection (156 mg/ml), were used to measure hematocrit rate and viscosity on a low-shear viscosimeter. All viscosity values are summed in Table 1. The hematocrit rate in both samples was evaluated at 45% the first day and 44% the day after. As shown in Table 1, blood viscosity, tested at different shear stresses, did not significantly change before and after TMP injection ($p > 0.4$ for each shear rate).

8. In vivo backscattering quantification

In order to work with an optimal signal to noise ratio, particles were injected in the in vivo experiments at a concentration of 7.8 mg/ml, corresponding to the maximal concentration within the linear zone according to the dose study on the Couette device. For all in vivo studies, a control group with injection of physiological serum was used to ensure that, after particle injection, potential IB enhancement quantification or improved intratumoral vessels detection was not induced by the use of the needle during the injection into the sinus-retro orbital vein. In this group, we could notice no IB enhancement from Doppler signals and we could detect the exact same intratumoral vessel number in the power Doppler mode before and after physiological serum injection.

8.1. Backscatter and velocity parameters

When measured on the aorta, a spectral Doppler enhancement was seen in all mice. Doppler measurements pre- and post-echo-contrast injection are represented on Fig. 3. The integrated intensity of the power spectra increased of 7.29 ± 1.72 dB ($p < 0.017$) over the first 4.5 min following particle injection. This value started decreasing significantly after 10.5 min but Doppler enhancement was still measurable after 12 min with a relative IB of 4.5 ± 0.83 dB after particle injection showing a very good stability of the TMP. The IB enhancement is thus equivalent to what was observed on the flow phantom with particles suspended in blood medium. On the aorta, the mean velocity extracted from the Doppler spectra and corresponding to the mean frequency did not change signif-

Table 1
Viscosity values measured on the micro-viscosimeter at different shear rates from 0.519 to 241 s^{-1}

		Shear rate (s^{-1})					
		0.519	2.41	11.2	51.9	96	241
Serum	Mean viscosity (cP)	4.3575	5.7925	6.38	12.3475	22.925	47.29
	SD	0.56251667	0.72766178	1.86588674	1.41419883	3.85978842	12.3883938
Particles	Mean viscosity (cP)	4.455	6.035	7.45	12.28	21.87	36.193333
	SD	0.44071911	0.68344714	0.88900694	1.4822437	4.81078649	8.17783794

Viscosity is expressed in cPoiseul and SD means standard deviation.

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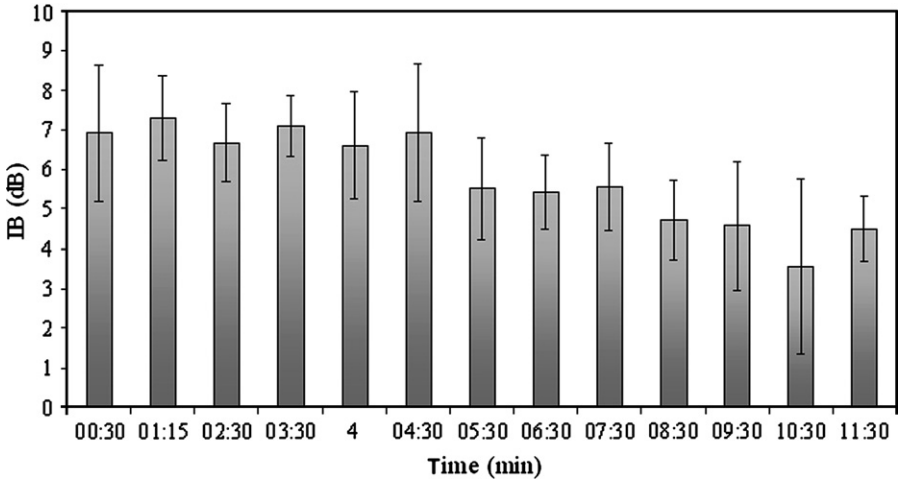

Fig. 3. Mean IB measured on an aorta at different times after particles injection (errors bars indicating the average within all experiments).

icantly after particle injection (1.204 ± 0.0058 cm/s versus 1.237 ± 0.034 cm/s).

8.2. Intratumoral vessel number quantification

For this quantification, tumors were entirely scanned in the power Doppler mode according to the longitudinal and transversal axis before and after particle injection. Usually, tumors already showed few vessels in power Doppler mode before injection of TMP depending on their volume. Indeed, we could find a strong correlation ($p < 0.001$) between tumoral volume and pre-injection intratumoral vessel number. After 0.1 ml bolus injection (156 mg/ml suspension), increased vessel number could be observed (Fig. 4) and quantified in all mice from 24 ± 3.75 to 38 ± 6.8 ($p = 0.007$). These additional detectable vessels

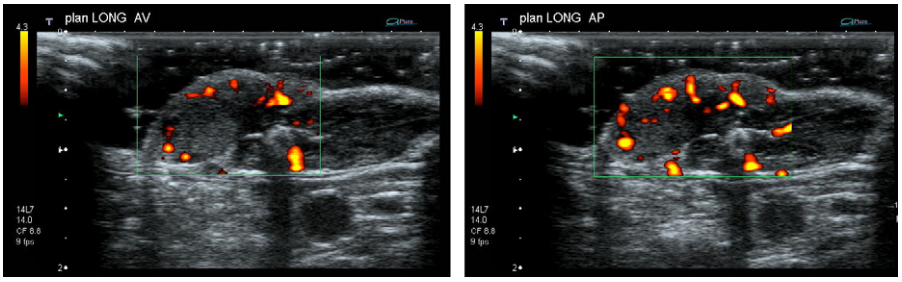

Fig. 4. Examples of intratumoral vessels observed in melanoma tumor scans in Doppler power mode before and after trisacryl particles injection.

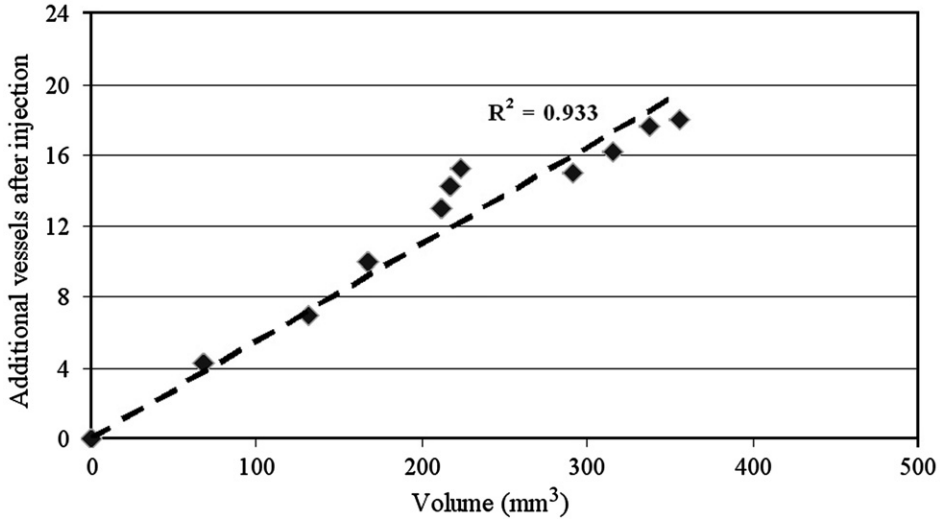

Fig. 5. Additional intratumoral vessels according to tumoral volume. Spearman Correlation coefficient: $R^2 = 0.933$, $p = 0.002$ for 13 mice.

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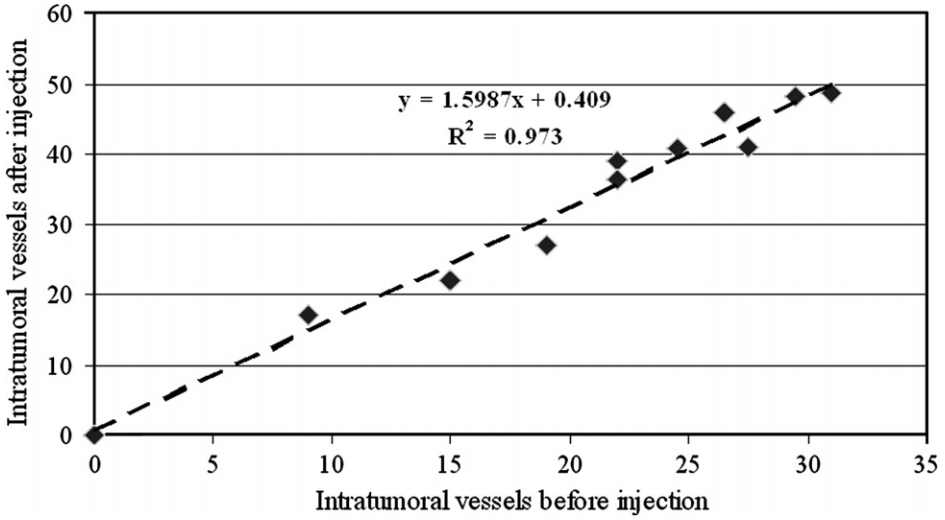

Fig. 6. Additional detected intratumoral vessels according to pre-injection intratumoral vessels number. Spearman correlation coefficient: $R^2 = 0.973$, $p < 0.0001$ for 13 mice. Linear regression in dash line with a slope of 1.598.

were graphically represented according to the tumoral volume (Fig. 5) and to the pre-injection detectable vessel number (Fig. 6) with a strong correlation to both parameters (Fig. 5: $R^2 = 0.933$ with $p = 0.002$ and Fig. 6: $R^2 = 0.973$ with $p < 0.001$). On Fig. 6, the slope of the linear regression allowed to quantify an improvement of 60% of the intravascular detectability after TMP injection.

9. Preliminary toxicity study

Injected mice exhibited neither side effect nor clinical sign of toxicity before sacrifice. Mice liver, kidneys, heart, lungs and spleen were analysed on HES colored microscopic slices after 1 h, 8 days and 16 days after TMP injection. At each sacrifice time, no inflammation phenomena induced by the MP was observed in the liver, the kidneys, the heart and the spleen. However, some particles could be detected inside lung vessels as soon as 1 h after injection. Still on the lung slices, macrophagic infiltrates surrounding the particles were observed at D8 and inflammation reaction increased at D16 with the additional presence of lymphocytes. However, particles entrapped inside the lungs seemed to stay inert over time.

10. Discussion

The objective of this paper was to evaluate the ultrasound integrated backscatter of new trisacryl contrast particles. This parameter was studied first in an in vitro phantom set-up developed to work under similar but controlled in vivo flow conditions and secondly, in nude mice. The acoustic response was measured simultaneously with a reference method (Doppler signal analysis) and with a technique more readily available in clinical routine (pulsed and power Doppler modes).

Most in vitro experimental works with ultrasound contrast particles are usually performed following dilution of particles in saline or other simple aqueous media. Since clinical applications, however, involve particle dilution in blood, it appeared interesting to study the acoustic properties of the TMP, not only in an usual reference medium (glycerol/physiological serum) but also in whole blood. Indeed, in vivo, contrast agents are suspended in a fluid containing a high volume fraction of cells of comparable size with contrast particles and the many differences between blood and saline including density, viscosity and microscopic structure (cells and other bodies) can potentially alter observed physical properties notably backscatter measurements as compared to in vitro observations. Phantom experiments on whole blood allowed indeed to anticipate in vivo acoustic behaviour of the trisacryl particles. These experiments actually revealed a maximal integrated backscatter IB of 7.5 ± 0.7 dB in a blood medium while IB in the glycerol/physiological serum was of 17 ± 0.88 dB. This observation was further confirmed in vivo when quantifying in pulsed Doppler the IB on the aorta with a maximal and constant enhancement of 7.29 ± 1.72 dB over the first minutes after TMP injection. According to the phantom experiments on whole blood, this expected decrease could be partially explained by the presence of red blood cells, shadowing the contrast effect of the particles.

Both in the phantom device and in vivo, the quantitative analysis of the TMP was performed with the audio component of Doppler signals extracted from an ultrasound clinical system, as they have been shown to correlate strongly with the relative contrast concentration and induced enhanced-backscattering [13,14]. This methodology based on the uncompressed signals allowed to compare the in vitro and the in vivo IB measurements. As contrast

agents are also known to enhance visualisation of Doppler signals in more applied clinical modes such as power or color Doppler modes, the TMP echo contrast was also studied through the enhancement of intratumoral vascularisation detection in power Doppler mode. This more qualitative but proved methodology, revealed a significant 60% enhancement after the TMP injection. However, as already described in our previous paper (Lavisse et al. Part I), the trisacryl agents, although deformable, seemed not flexible enough to generate harmonic frequencies under different acoustic pressure conditions. Until we use more flexible particles (work in progress), enhancement measurements can not be investigated in harmonic modes working at low mechanical indexes.

Using a custom-made silex 10-MHz cuff transducer placed directly around a surgically exposed vessel, Forsberg et al. measured also in vivo dose-response from audio Doppler signals. With surfactant ST68 and polymeric PLGA particles, a maximum enhancement of 18 and 20 dB was respectively measured at a 0.13 and 0.15 mg/ml concentration injected in rabbits [16,17]. Thus, given the current particle conformation, the TMP have shown so far relatively limited interest in enhancing echo contrast in vivo compared to other studied particles. To extend their applications however, these particles are currently investigated to contain a more flexible membrane and specifically target tumoral vessels in order to increase backscattered signals by accumulation at the tumoral site.

Another aspect explored as a preliminary study in this paper concerns the potential toxicity of the TMP. At D8 and D16 after their injection into the retro-orbital vein of mice, particles appeared to stay especially in the lungs. On the corresponding slices, they seemed to form aggregates with exceeding diameters to pass through the capillary bed. In this tissue, particles remained unchanged for up to 2 weeks and initiated a polynuclear reaction. Aggregates could find their origin from the high particle density in the 156 mg/ml suspension before intravenous (i.v.) injection and they could thus be filtered by lungs after administration. Their presence over time inside pulmonary capillaries could be expected as acrylate particles have a rather nonresorbable nature and it had already been observed by Laurent et al. [18] with gelatin trisacryl microspheres (200–1000 μm) injected in order to embolise pulmonary arteries and found in the lung over nine months after injection. Moreover, Sonavist contrast agent (SHU 563A) developed by Schering (AG, Berlin) and composed of cyano-acrylate monomers, was also observed to remain for extended durations in patients in the Knüpffer cells after phagocytosis by the reticuloendothelial system [19]. However, in each case, the particles were inert and induced no toxicity.

However, both from an acoustic and safety points of view, these TMP have to be further developed to modify their relatively solid membrane by decreasing as a first step the MBA tensio-active proportion (in progress). This modification, along with the TMP deformability potential, could on one hand enable the particles to resonate and generate harmonic frequencies, and on the other hand improve their in vivo safety and elimination process.

11. Conclusion

By analysing the audio component of the Doppler signal, the present study demonstrated the acoustic effect of trisacryl microparticles in a home-made flow phantom and on mice aorta with pulsed-wave Doppler US. It was shown that i.v.-administered TMP were effective in significantly increasing Doppler signal intensity at the peak of the contrast effect compared to pre-contrast (7.29 ± 1.72 dB over the first minutes). This was associated with a significant increase in the intratumoral vascularization detection (+60%) as determined from power Doppler mode. To optimize these particles, tumoral targeting experiments are currently in progress first to specifically improve TMP enhancement in the tumoral vessels and secondly, to deliver associated chemotherapeutic agents [20]. Moreover, TMP have been fluorescently labeled (DAFT labeling) to further investigate their biodistribution in the different tissues.

Acknowledgments

We sincerely thank Biosphere Medical and especially Philippe Reb and Céline Chaix for providing the trisacryl microparticles. The authors are also grateful to the Animal Department (SCEA) for animal care, to Muriel Del Pino for the viscosity measurements and to Elisabeth Connault for histological slices preparation.

Finally, authors warmly thank the Agence Nationale de la Valorisation de la Recherche (ANVAR) for financial support.

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Please cite this article in press as: S. Lavisse et al., Acoustic characterization of a new trisacryl contrast agent. Part II: ..., Ultrasonics (2008), doi:10.1016/j.ultras.2007.10.006