Release of cardiac bio-markers during high mechanical index contrast-enhanced echocardiography in humans

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Received 8 September 2006; revised 19 February 2007; accepted 1 March 2007; online publish-ahead-of-print 4 April 2007

See page 1190 for the editorial comment on this article (doi:10.1093/eurheartj/ehm110)

Introduction

Current ultrasound (US) contrast agents typically consist of a suspension of gas microbubbles that have been specially designed to interact with diagnostic US and produce image enhancement.1 Because these microbubbles are compressible, they alternately contract and expand in the acoustic field, a phenomenon which is referred to as cavitation. At low acoustic pressure, the microbubbles usually grow and shrink rhythmically and symmetrically around their equilibrium size. This phenomenon is known as a stable or non-inertial cavitation. At higher acoustic pressure, the expansion and contraction of the microbubbles usually become unequal and markedly exaggerated, leading to their destruction.2–4 This second form of activity is known as inertial cavitation.

There is accumulating evidence that the process of cavitation, and particularly inertial cavitation, may produce microscale damage to organs containing air, such as the lungs,5 or exposed to US contrast agents, such as demonstrated ex vivo, in isolated rabbit hearts,6 and in vivo, in the mouse,7 rat,8–11 and dog myocardium.12 Studies in rodents have identified specific combinations of contrast dose, US pressure, delivery mode, and duration of US exposure below which these bio-effects seldom occur, and above which they are almost invariably observed.7–11

Translation of observations made in the experimental lab to the clinical situation of patients undergoing contrast-enhanced echocardiography is by essence difficult. Based on the results of the published multicentre trials,13–16 it would seem that the use of US contrast agents in humans is safe, the only side effects reported in these trials being a slightly increased incidence of premature ventricular contractions. Despite of these re-assuring data, post-marketing surveillance reports have recently indicated an unusually high incidence of serious adverse events in high risk coronary patients undergoing left ventricular opacification with one of the commercially available US contrast agents. This has prone the EMEA to modify the indications for use of this agent, forbidding it in the higher risk groups.17

In view of the persisting uncertainty as to the clinical relevance of the microscale bio-effects seen in animal models during contrast-enhanced echocardiography, we designed the present study to investigate whether high mechanical index (MI) contrast-enhanced echocardiography can cause subclinical release of cardiac bio-markers in humans, while low-MI real-time imaging appears to be safer.

Methods

Patient population

Over a period of 6 months, all patients who were referred to our Institution for diagnostic coronary angiography were prospectively screened for possible inclusion into this study. Inclusion criteria

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were a stable clinical condition, a normal global and regional left ventricular function, a body mass index <30, good 2D-echocardiographic images from the apical window, and a normal renal function. Of the 33 screened patients who fulfilled the above inclusion criteria, 20 (14 males, mean age 60 ± 12 years, range 38–78 years) accepted to participate into this study and gave their written informed consent. The study protocol was approved by the Ethical Committee of our institution.

**Myocardial contrast echocardiography**

Perfluorocarbon-enhanced Sonicated Dextrose Albumin (PESDA), a second-generation contrast agent, consisting of decafluorobutane-filled albumin microbubbles with mean diameter of 4.2 ± 0.5 μm and a mean concentration of 10^8 mL⁻¹ (as determined with a Coulter Multisizer Z2 Analyzer, Accucomp Software) was used in this study. PESDA was infused at a rate of 0.01 mL kg⁻¹ min⁻¹ during 15 min.

Imaging was performed from the apex with a SONOS 5500 system (Philips Medical System, Andover, MA, USA) equipped with a broadband wide-angle phased array 53 transducer (1–3 MHz). Imaging was performed either in a low or a high-MI mode. Low-MI imaging was performed in real time (20 frames/s) using power modulation and a MI of 0.2. In patients imaged with this mode, 10 consecutive high-MI frames (MI of 1.7) were transmitted on demand every minute. Imaging then returned automatically to low-MI real-time scanning. High-MI imaging was performed in triggered (one frame every other beat) second harmonic mode, using a transmit frequency of 1.3 MHz and a MI of 1.5. For a patient whose heart rate would have been around 60 beats per minute, a total of approximately 450 high-MI frames would have been transmitted.

**Coronary sinus and arterial catheterization**

Before contrast echocardiography and coronary angiography, a coronary sinus catheter (Medtronic, USA) was inserted via the right femoral vein into the coronary sinus for withdrawal of coronary sinus blood samples. Appropriate positioning of the coronary sinus catheter was always confirmed by use of iodinated contrast injections. An arterial catheter was also inserted into the right femoral artery for simultaneous withdrawal of arterial blood samples. Coronary sinus and arterial blood samples were obtained at several time points: at baseline (before US exposure), as well as at 3 and 15 min after the start of the imaging protocols.

**Laboratory analysis**

Heparinized coronary sinus and arterial blood samples were obtained at the several time points during the experiments for determination of lactate, myoglobin, creatine kinase, CK-MB, troponin I, and oxygen content. Myoglobin was determined using the Access Myoglobin immunoassay (Beckman Coulter ™, USA; reference range: 1–65 ng/mL; coefficient of variance: 5.9%), cTnI using the Access AccuTnl immunoassay (Beckman Coulter ™, USA; reference range: <0.06 ng/mL; coefficient of variance: 8.6%) and CK-MB mass using the Access CK-MB immunoassay (Beckman Coulter ™, USA; reference range: 0.5–3.5 ng/mL; coefficient of variance: 8.6%). CK activity (reference range for woman: <400 UI/L; reference range for man: <200 UI/L; coefficient of variance: 1.5%), and lactate (reference range: 0.9–1.7 mM, coefficient of variance: 2.2%) were determined using assays from BioMérieux, France. All the measurements were done in duplicate.

**Study protocol**

The echocardiographic studies were completed before coronary angiography. The patients were randomly assigned to one of three groups by ‘drawing of lots’: a ‘high-MI-no-contrast’ control group, a ‘high-MI-contrast’ group, and a ‘low-MI-contrast’ group. Patients from the control group were imaged according the high-MI imaging protocol for 15 min but did not receive any contrast agent. Patients from the ‘high-MI-contrast’ and the ‘low-MI-contrast’ groups both received a continuous intravenous infusion of PESDA and were imaged according to either the high-MI or the low-MI imaging protocols for 15 min. For obvious reasons, the investigators were not blinded to the results of the randomization procedure. Coronary sinus and arterial blood samples were obtained at baseline (before US exposure), as well as at 3 and 15 min after the start of the imaging protocols.

**Statistical analysis**

Values are expressed as means ± SD. For continuous variables, groups were compared at each time point using the Kruskal–Wallis test. Individual comparisons between groups were evaluated post hoc using the Mann-Whitney test. For categorical variables, comparisons between the groups were performed using the Fisher exact test. A P-value less than 0.05 was considered indicative of a statistically significant difference.

**Results**

**Patient characteristics**

Patient characteristics are summarized in Table 1. No significant differences in baseline characteristics were found among the three groups. During the experiments, no
patient experienced any side effects, symptoms, or ST-segment changes.

Haemodynamic parameters

Heart rate, systolic blood pressure, and the rate-pressure product (Figure 1) were similar at baseline among the three groups and did not vary significantly over time. Baseline coronary sinus blood oxygen saturation was also similar among the three groups (26 ± 9% in the control group, 26 ± 4% in the low-MI group, and 28 ± 7% in the high-MI group) and remained stable throughout the experiments (Figure 2).

Myocardial bio-markers

At baseline, the coronary sinus blood concentrations in lactate, CK, CK-MB, myoglobin, and troponin I were found to be similar in the three groups, as were the baseline arterio-venous differences in these parameters. During insonation, the arterio-venous differences in lactate and CK concentrations did not change significantly over time in neither of the three groups (Figures 3 and 4). Although the arterio-venous difference in CK-MB, myoglobin and troponin I also remained stable in the control and Low-MI-contrast groups, it progressively increased over time in the High-MI-contrast group, albeit not significantly so as far as myoglobin is concerned. Figures 5, 6, and 7 illustrate the time course of myoglobin, CK-MB, and troponin I arterio-venous differences, respectively, among the three groups.

Figure 1 Bar graph showing the time course of rate-pressure product in the three patient groups. Closed bars: control group; open bars: low-MI-contrast group; Gray bars: high-MI-contrast group.

Figure 2 Bar graph showing the coronary sinus oxygen saturation in the three patient groups before and at the end of the study protocol. Closed bars: control group; open bars: low-MI-contrast group; Gray bars: high-MI-contrast group.

Figure 3 Graph showing the time course of the arterio-venous difference in lactate concentration. Closed circles: control group; open circles: low-MI-contrast group; closed triangles: high-MI-contrast group.

Figure 4 Graph showing the time course of the arterio-venous difference in total CK concentration. Closed circles: control group; open circles: low-MI-contrast group; closed triangles: high-MI-contrast group.

Figure 5 Graph showing the time course of the arterio-venous difference in myoglobin concentration. Closed circles: control group; open circles: low-MI-contrast group; closed triangles: high-MI-contrast group.
Discussion

The aim of the present study was to investigate whether the combined exposure of patients to contrast-enhanced echocardiography results in any detectable cardiac bio-effects. For this purpose, we chose to use the currently most sensitive test for detection of myocardial damage, i.e. the measurement of the arterio-venous differences in cardiac bio-markers. Our results indicate that the combined exposure of patients to contrast-enhanced echocardiography increases the circulating levels of cardiac markers including myoglobin, lactate, and total CK. It is interesting to note that only the most sensitive and specific marker of myocyte damage, i.e. cTnI, was found to consistently increase. There are several plausible explanations for this observation. First, cTnI is normally not present in the peripheral blood. Thus, the signal to noise ratio is more favourable for the detection of myocardial damage with cTnI than for any of the other markers. Also, the assay used in this study can detect the binary complexes troponin-I/troponin-C and troponin-I/troponin-T, the ternary complexes troponin-I/troponin-C/troponin-C, as well as the phosphorylated and the non-phosphorylated forms of these complexes. All these reasons can explain why the increase in cTnI reached statistical significance, both at 3 and 15 min after the start of US exposure, whereas it merely became statistically significant at 15 min for CK-MB and was never significant for the other markers, including myoglobin, lactate, and total CK.

The mechanism by which myocytes are damaged during contrast-enhanced echocardiography cannot be deduced from our study. Previous works in cultured cells and in small animals have suggested that cell damage can be produced by at least two independent mechanisms: a direct mechanism (hydrodynamic shear stress), leading to cell membrane perforation or rupture, and an indirect mechanism, most probably ischaemic in origin, due to microvascular destruction and obstruction. Although the present study was not designed to investigate the mechanism of the observed damage, the time course of troponin release in the coronary sinus is more consistent with a direct, possible mechanical toxicity than with ischaemic changes.

Clinical implications

Contrast agents are now frequently used to improve image quality in patients with poor acoustic windows and are investigated as a means to evaluate myocardial perfusion. The present study demonstrates that the combined exposure of the heart to high-MI US and US contrast agents, in the MI and dose range of clinical contrast-enhanced echocardiography, can induce microscale myocyte damage, possibly raising concerns as to the safety of this technique. Until now, the only significant cardiac side effects reported during high-MI contrast-enhanced echocardiography were the appearance of premature ventricular contractions. In a study performed in healthy male volunteers, van Der Wouw et al. indeed reported the occurrence of premature ventricular contractions during high-MI contrast-enhanced echocardiography when using end-systolic (but not end-diastolic) triggering. These authors also noted that the frequency and magnitude of these side effects were dependent on the dose of US contrast agents. More recently, Hayat et al. reviewed the safety results of a large number of multicentre trials that have investigated the safety of US contrast agent administration in patients undergoing contrast-enhanced echocardiography. These authors found no evidence, whatsoever, of clinically relevant side effects. Finally, Borges et al. examined if contrast-enhanced echocardiography increased the circulating levels of cardiac markers and found, like in our study, that their peripheral blood concentration was not affected.
From all these previous studies, it would thus seem that the use of US contrast agent, in combination with echocardiography, is safe and that the microscale toxicity noted in our study is most probably clinically irrelevant. In this regard, it should nonetheless be noted that most of the studies performed so far have recruited patients in stable clinical conditions and usually with a normal left ventricular function. There are currently no data to indicate that contrast-enhanced echocardiography can be safely performed in patients with ongoing myocardial ischaemia or infarction or in those who are haemodynamically unstable. On the contrary, post-marketing surveillance reports have indicated that use of contrast-enhanced echocardiography in these patients could be associated with an increased incidence of serious adverse events, including death.17 Although these events were retrospectively considered as possibly allergic in origin, we cannot exclude the possibility that microscale myocyte damage also contributed. We therefore believe that contrast echocardiography should be used with caution in these patients by avoiding imaging at high MI and using the lowest dose possible of US contrast agents.

Limitations of the study

First, in this study, the contention that high-MI contrast-enhanced echocardiography results in the net release of cardiac bio-markers in the coronary sinus is solely based on the observation of directional changes in the arterio-venous concentration differences in these markers. Unfortunately, the directional changes in the arterio-venous concentration differences and the net release or uptake of any marker by any organ, including the heart, do not necessarily run in parallel. Net release or uptake of a marker by any organ is indeed the product of flow through that organ and the arterio-venous concentration difference in the marker of interest. Changes in tissue perfusion can thus be an important confounding factor. Since in the present study, we did not measure myocardial blood flow, the validity of our conclusion relies on the assumption that myocardial blood flow did not change during the experiments. The fact that the rate-pressure product and more importantly the coronary sinus oxygen saturation remained stable throughout the experiments supports this assumption. Secondly, we only used PESDA and no other US contrast agent in this study. Our results cannot therefore be generalized to other, commercially available, contrast agents. However, significant differences between US contrast agents in terms of toxicity are unlikely in view of the lack of agent specificity noted in experimental studies.6,24 Also, the dose of PESDA used in our experiments was relatively large. It was nonetheless within the dose range used and reported in the literature.12,25–28 as well as within that used daily in our lab during stress perfusion studies. The fact that, when present, attenuation artefacts were confined to the mitral annulus level and did not extend significantly into the LV cavity supports our contention that the dose of contrast used in our study was within the clinically relevant dose range. The absence of a priori sample size calculation and the relatively small number of patients finally enrolled in this study (6–7 patients per group) could also be viewed as a limitation. However, to calculate the sample size needed to reach statistical significance, we should have known the normal range of coronary sinus concentration and arterio-venous differences for the different bio-markers measured in our study. Unfortunately, these data are not available in the literature. We therefore decided to analyse the data after having recruited seven patients in the high-MI contrast group. Because a statistically significant toxic effect was already demonstrated in six of these patients, we felt it unethical to continue enrolling patients in that group.

Conclusions

Our data demonstrate that high-MI contrast-enhanced echocardiography can cause subclinical myocardial bio-markers release in humans in the dose range where both US contrast agents and US acoustic pressure are used in daily clinical practice. On the other hand, our results indicate that low-MI real-time contrast-enhanced echocardiography is probably safe, as it does not result in any significant release of the same cardiac bio-markers.

Acknowledgements

This work is supported in part by Grant nos 3-4563-98 and 3-4504-03 from the ‘Fonds National de la Recherche Scientifique et Médicale and by the ‘Action de Recherche Concertée’ no. 01/06-271. D.V. is supported by the ‘St-Luc’ and the ‘Damman’ Foundations, Louvain-la-Neuve, Belgium.

Conflict of interest: none declared.

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