Research Paper

Journal of the Royal Society of Medicine Cardiovascular Disease
2015, Vol. 4: 1–6

DOI: 10.1177/2048004015601564
cvd.sagepub.com

Microbubbles shunting via a patent foramen ovale impair endothelial function

Henry Fok[2], Benyu Jiang[2], Phil Chowienczyk[2] and Brian Clapp[1]

Abstract

Objectives: Exposure to intravascular microbubbles after diving and during medical procedures alters endothelial function. The aim of this study was to investigate whether a patent foramen ovale altered forearm endothelial function by facilitating microbubbles transfer.

Design: Patients attended on two separate visits, at least seven days apart receiving agitated saline or no active intervention in random order. On both days, flow-mediated dilatation of the brachial artery was measured using vascular ultrasound. On the intervention visit, agitated saline was injected and the passage of microbubbles into the arterial circulation was confirmed by echocardiography. Serial flow-mediated dilatation measurements were made after agitated saline and at the same time points after no intervention.

Setting: St Thomas' Hospital in London.

Participants: Patients with a patent foramen ovale (PFO+n = 14, 9 male, mean ± SD age 42.2 ± 10.5 years) and patients without a patent foramen ovale (PFO− n = 10, 7 male, mean ± SD age 49.4 ± 18.4 years) were recruited.

Main outcome measures: Change in brachial artery flow-mediated dilatation.

Results: In patent foramen ovale + patients, flow-mediated dilatation did not change significantly on the control day but after agitated saline reduced by 2.3 ± 0.3%, 20 minutes after bubble injection ($P < 0.005$ vs. corresponding change in flow-mediated dilatation during control study). There was no significant change in flow-mediated dilatation for patent foramen ovale− patients at either visit.

Conclusion: These results suggest that the presence of a patent foramen ovale facilitated impairment of endothelial function acutely by the transfer of microbubbles into the arterial circulation. As a patent foramen ovale is a common condition, this may be relevant to microbubbles exposure in medical procedures and in decompression illness.

Keywords

Decompression illness, endothelial dysfunction, flow-mediated dilatation, microbubbles, patent foramen ovale

Introduction

Formation of microbubbles within the circulation occurs after diving and during medical interventions such as haemodialysis and cardio-pulmonary bypass and may have deleterious effects on the endothelium and other tissues.[1–5] Decompression illness, for example, is thought to occur when microbubbles formed as a result of inert gas supersaturation under hyperbaric conditions encountered during diving are released in high quantity upon rapid decompression.[1,2,6] In most instances, microbubbles are filtered by the pulmonary circulation and expired via the lungs thereby preventing the passage of microbubbles into the arterial circulation. Excessive numbers of venous microbubbles are thought to be able to 'swamp' this pulmonary filter and pass unchecked into the arterial circulation where their amplification in inert gas saturated tissues leading through endothelial dysfunction to clinical decompression illness.

The presence of an inter-atrial shunt such as a patent foramen ovale (PFO), which is present in 27% of the general population,[7] could potentially bypass the pulmonary filtering mechanism hence explaining the higher incidence of decompression illness in patients with as compared to those without a PFO.[8–11] Furthermore, in medical procedures where exposure to microbubbles is common, such as during

[1]Guy's and St Thomas NHS Foundation Trust Hospital, London, UK
[2]King's College London, British Heart Foundation Centre, London, UK

Corresponding author:
Brian Clapp, Department of Cardiology, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, UK.
Email: brian.clapp@gstt.nhs.uk

cardiopulmonary bypass, the presence of a PFO could potentially exacerbate conditions such as post cardiopulmonary bypass neuropsychological syndrome by similar mechanisms.[1,3,4]

The aim of the study was to investigate whether the passage of microbubbles through a PFO into the arterial circulation at atmospheric pressure leads to a change in flow-mediated dilatation (FMD) – a marker of endothelial function. Serial measurements of brachial artery FMD were made at baseline and at regular intervals following intravenous administration of agitated saline in patients with and without a PFO. We hypothesised that patients with a PFO would have a significant reduction in FMD following exposure to agitated saline.

Methods

Patients with a known PFO (PFO+, n = 14, 9 men, mean ± SD age 42.4 ± 10.4 years) and those without a PFO (PFO−, n = 10, 7 men, age 49.4 ± 18.5 years) were recruited from those previously investigated by bubble contrast echocardiography at Guy's and St Thomas' Hospital. Subject characteristics and indications for bubble contrast echocardiography are shown in Table 1. Patients were excluded if they had intercurrent illness, pregnancy, personal history of valvular heart disease, echocardiographic evidence of left ventricular ejection fraction < 50%, regional wall motion abnormality, left ventricular outflow tract obstruction, pulmonary hypertension or a poor transthoracic acoustic window. The study was approved by the London Westminster research ethics committee and the research was carried out in accordance with the Declaration of Helsinki (2008) of the World Medical Association. All patients gave written informed consent. An initial time course study was performed in PFO + patients. Both PFO+ and PFO− patients then participated in an unblinded crossover study receiving no active intervention and agitated saline injection in random order on two occasions at least seven days apart. Studies were performed in a quiet temperature controlled (24–26°C) laboratory and all patients were asked to avoid tobacco, caffeine and alcohol on the day of the study.

Time course study in PFO+ patients

PFO+ patients (n = 10) participated in this study. On arrival, a peripheral venous catheter was inserted into the left antecubital fossa. After 30 minutes of resting supine baseline endothelial function was assessed by brachial artery FMD as described below. Following successful acquisition of baseline FMD measurements, a marker was placed over the skin where the linear transducer ultrasound probe was located to ensure repeat FMD measurements were taken at the same segment of the brachial artery. Agitated saline injection was then performed during echocardiography using

Table 1. Baseline characteristics.

	PFO-positive patients (n = 14)	PFO-negative patients (n = 10)
Age (years)	42.1 ± 10.1	49.4 ± 18.5
Sex (M:F)	9:5	7:3
Baseline systolic blood pressure (mmHg)	129 ± 9.35	130 ± 12.5
Baseline diastolic blood pressure (mmHg)	78.7 ± 9.21	76.3 ± 10.7
Body mass index (kg/m^2)	25.6 ± 3.08	28.8 ± 3.62
Total cholesterol (mmol/L)	4.67 ± 0.865	5.47 ± 0.592
High-density lipoprotein (mmol/L)	1.68 ± 0.324	1.67 ± 0.407
HbA1c (%)	5.51 ± 0.430	5.64 ± 0.220
Diabetes mellitus (n)	1	0
Smoking (n)	3	2
Antihypertensives (n)	6	2
Statins (n)	6	2
Disease triggering original PFO investigation		
Cerebral infarction (n)	7	1
Decompression illness (n)	4	1
Migraine (n)	3	2
Myocardial infarction (n)	0	1
Research investigations (n)	0	5

PFO: patent foramen ovale.

the Vivid-7 ultrasound platform (General Electric Healthcare, UK) with the patient in the left lateral decubitus position. A four-chamber view was optimised to focus on the left and right atrium. Agitated saline, created by agitating a mixture of 0.5 ml of air and 8 ml of saline, was injected intravenously by a second operator (HF). Inter-atrial shunting of microbubbles was confirmed when more than five bubbles were demonstrated in the left atrium within seven cardiac cycles following complete opacification of the right heart. If shunting was not demonstrated agitated saline injection was repeated during a valsalva manoeuvre for 10 seconds before injection of agitated saline. Upon complete opacification of the right atrium the patient was asked to release the valsalva manoeuvre. Microbubble shunting was confirmed as described above. Up to a maximum of five valsalva manoeuvres with agitated saline injection were repeated and the procedure was stopped as soon as shunting was demonstrated. For PFO+ patients, an average of three valsalva manoeuvres with agitated saline injections was required to demonstrate inter-atrial microbubble shunting. The subject then returned to the supine position with the same right brachial artery segment imaged for FMD at 20, 40 and 60 minutes after demonstration of microbubble shunting.

Cross-over study in PFO– and PFO+ patients

All patients: PFO− (n = 10) and PFO+ (n = 14, including those in the time course study) participated in a cross-over study to control for possible confounding effects of repeated measures of FMD and to determine whether response to agitated saline injection was specific to PFO+ patients. FMD responses were assessed on two visits performed in random order separated by seven days. On visit 1 (no intervention), FMD was measured before and after no intervention or a valsalva manoeuvre (n = 5, PFO+ patients) without injection of agitated saline. On visit 2 (agitated saline), FMD was measured before and after injection of agitated saline and demonstration of microbubble formation in PFO+ patients as described in the time course study. PFO− status was confirmed when inter-atrial microbubbles shunting was not demonstrated following at least five agitated saline injections with valsalva manoeuvre. On both visits, FMD was measured at baseline and at 20 minutes after no intervention/agitated saline, this being the time at which changes in FMD were near maximal on the time course study above.

FMD

FMD was performed by an experienced operator (BJ) to assess endothelium-dependent vascular reactivity according to the recommendations of the International Brachial Artery Reactivity Task Force Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilatation of the brachial artery.[12] A high-resolution ultrasound system with a 7–10 MHz linear array transducer (Acuson Aspen, Acuson Corporation, Mountain View, CA, USA) was used to image a longitudinal section of the right brachial artery, 2 to 15 cm above the elbow. After visualisation of the artery, the transducer was secured in place by a stereotactic probe holder and a baseline scan is performed. Reactive hyperaemia was then induced by inflation of a pneumatic tourniquet placed around the forearm (distal to the arterial segment being scanned) to a pressure of 250 mmHg for five minutes, followed by release. The images were recorded continuously for one minute at baseline, five minutes during cuff inflation and five minutes post cuff deflation. Images were digitised for subsequent analysis. The diameter of the brachial artery was determined using semi-automated edge detection software (Brachial Analyser, Medical Imaging Applications, LCC, Iowa, USA). FMD was expressed as the percentage increase in brachial artery diameter from baseline to maximal dilation, which occurs in all patients at 30 to 90 seconds after release of the cuff.

Statistical analysis

Subject characteristics are summarised as means ± SD and results as means ± SEM. Differences in the responses to agitated saline injection relative to baseline and differences in responses between visit 1 and 2 were analysed by repeated measures analysis of variance. $P < 0.05$ was considered significant, and all tests were two tailed. Analysis was performed using SPSS version 19. A target sample size of $n \geq 10$ was set to provide 80% power to detect a change in FMD > 2% at a two-sided 5% significance level, based on a within subject SD of 1% in FMD.

Results

Time course study in PFO+ patients

For PFO+ patients participating in the time course study, FMD was significantly lower 20 minutes and 40 minutes after microbubble shunting and there was a trend for FMD to continue to be reduced at 60 minutes after microbubble shunting (2.2 ± 0.4%, 2.0 ± 0.7% and 1.2 ± 0.4% reduction in FMD at 20, 40 and 60 minutes after microbubble shunting respectively, n = 10, $P < 0.005$ Figure 1).

Crossover study in PFO- and PFO+ patients

In PFO− patients, there was no significant change in FMD after no intervention or after agitated saline injection (maximum reduction in FMD from baseline $0.11 \pm 0.18\%$ and $0.17 \pm 0.15\%$, respectively, each P = NS, Figure 2). In PFO+ patients, there was no significant change in FMD repeated 20 minutes after no intervention irrespective of whether a valsava manoeuvre was performed (reduction from baseline $0.59 \pm 0.57\%$ and $0.45 \pm 0.71\%$ for PFO+ patients not performing and performing valsalva manoeuvres respectively). By contrast, in these PFO+ patients, there was a significant reduction in FMD 20 minutes after saline injection and microbubble shunting by $2.3 \pm 0.3\%$ points ($P < 0.005$) representing a $38.6 \pm 5.8\%$ relative reduction in FMD (Figure 2). In all patient groups, brachial artery diameter immediately before reactive hyperaemia was similar on both study days and before and after saline injection.

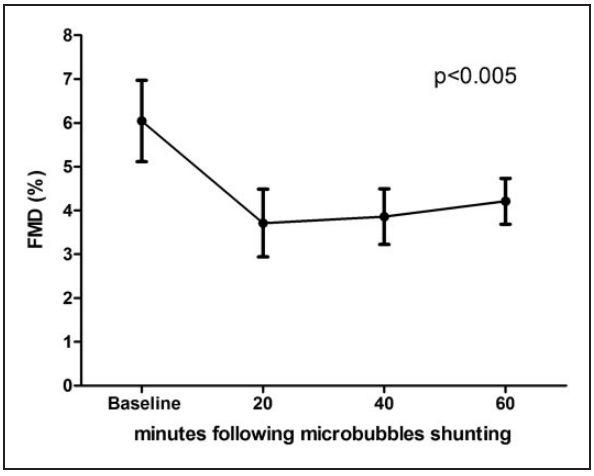

Figure 1. Brachial artery flow mediated dilatation in time course study for PFO+patients.
PFO: patent foramen ovale; FMD: flow-mediated dilatation.

Discussion

To our knowledge, this is the first human *in vivo* study demonstrating that the passage of microbubbles through a PFO acutely reduces nitric oxide-dependent endothelial function in a conduit artery. This effect is mediated by a relatively large number of bubbles rapidly accessing the arterial system and did not occur in the absence of an atrial level communication. Microbubbles had to be present for the impairment of endothelial function to occur and were not reproduced in control studies or subjects with a PFO in whom no agitated saline was used.

Systemic venous microbubbles occurring following diving and during medical procedures such as bypass surgery and haemodialysis are usually removed from the body by passage through the pulmonary circulation and expiration. The presence of an inter-atrial communication may allow this mechanism to be bypassed permitting the microbubbles to exert effects directly upon the arterial circulation. Animal studies have suggested that systemic arterial microbubbles may directly trigger acute endothelial dysfunction leading to a pro-inflammatory and pro-thrombotic state. However, this process is less well defined in human *in vivo* studies.[1,5] Coronary artery bypass surgery and haemodialysis have been associated with peripheral and central endothelial dysfunction.[13–15] This is believed to be a risk factor for vascular disease and the mechanism of the

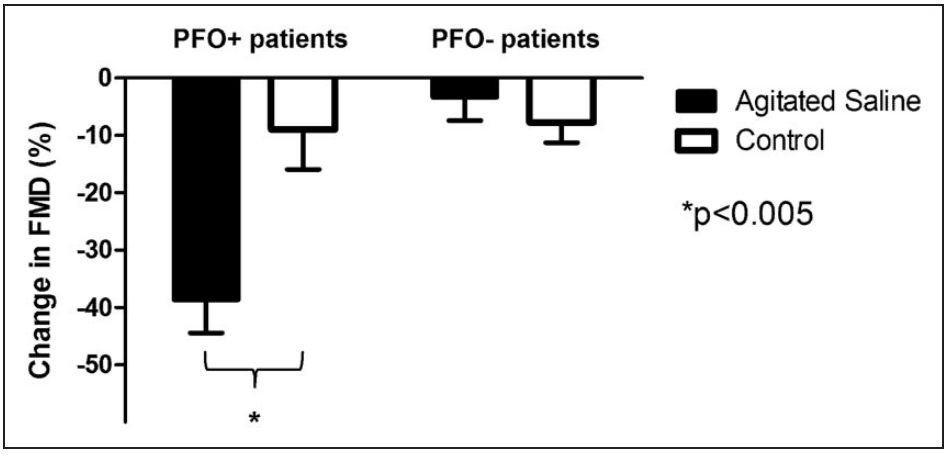

Figure 2. Relative reduction in brachial artery flow mediated dilatation at 20 minutes following agitated saline injection in PFO+ patients and PFO− patients.
FMD: flow-mediated dilatation; PFO: patent foramen ovale.

phenomenon has not been fully characterised. In the context of scuba diving, it has been shown that a reduction or impairment of endothelial function occurred following a single dive, which was prevented by pretreatment with antioxidants such as Vitamin C.[16–19] More recently, Vince et al.[16] detected a significant rise of VCAM-1 associated endothelial microparticles following a single dive in humans suggesting endothelial activation and/or apoptosis.

The current study aimed to explore the effects of injected microbubbles in order to study the time course of any outcome in a controlled environment. This model more closely equates to medical interventions than decompression illness where the supersaturation of all the tissues may be relevant to the pathophysiology of the condition. Within this protocol, the studies were performed under normobaric, normoxic condition, and this might explain the smaller degree and relatively short-lived alteration in endothelial function when compared to previous studies carried out under hyperbaric conditions with tissue supersaturation of microbubbles.[20] However, the current study cannot infer that a similar rapid recovery would occur in the setting of nitrogen-saturated peripheral tissues as found following diving. Since FMD is thought to be mediated through release of endothelium nitric oxide synthase (eNOS) derived nitric oxide (NO),[21] microbubbles may have a direct or indirect effect on NO bioavailability. The current study cannot determine whether the reduced bioavailability of NO is through reduced production, increased inactivation, a diminished response to available NO or a combination of these. In animal models, however, microbubbles have been shown to mediate a direct mechanical damage on the microvasculature and also induce acute endothelial dysfunction through the effects of increased oxidative stress on the vascular endothelium leading to activation of leucocyte, complement and clotting cascade systems.[1]

There are a number of limitations to our study, which are particularly relevant when considering it as a model of decompression illness, although less so in the setting of medical interventions. FMD is a marker of conduit artery rather than microvascular endothelial function and it is likely that the latter is the predominate site of abnormalities in some conditions, particularly such as decompression illness. Although impairment of FMD usually occurs in parallel with microvascular endothelial dysfunction, this cannot necessarily be inferred from the present study. The present studies were performed without prior hyperbaric conditions and therefore cannot mimic the inert gas supersaturation at the tissue level that occurs with diving and further studies are required to investigate the interaction between of microbubbles in human vascular endothelium in this setting. Due to the nature of the individuals undergoing clinical investigation for inter-atrial shunts, some subjects in these studies had established vascular risk factors. It is unlikely that these altered the results as similar effects were seen in all subjects and the cross-over nature of the protocol ensured individuals acted as their own control, however, this cannot be excluded.

In conclusion, this study shows that the passage of microbubbles into the arterial system through a right to left shunt at the atrial level leads to an acute deterioration in conduit artery endothelial function. As PFO is common in the general population, the results of our present study may have significant implications for understanding vascular function following diving and medical procedures involving exposure to microbubbles such as during cardiopulmonary bypass and haemodialysis. Further studies are needed to examine the mechanism of these pathological effects of microbubbles on the microcirculation and possible interventions that may protect against microbubble-induced endothelial dysfunction.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This work was supported by a British Heart Foundation Clinical Research Training Fellowship [FS/11/11/28634] to Dr Fok. The authors also acknowledge financial support from the Department of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust.

Ethical approval

None.

Guarantor

The guarantor for this study is Dr Brian Clapp.

Contributorship

Dr Brian Clapp (BC) conceived the study question and he was the principal investigator for this study. Dr. Henry Fok (HF) and Dr Benyu Jiang (BJ) were involved in data collection. BC, HF and Professor Philip Chowienczyk (PC) were involved in the design, analysis, results interpretation and manuscript preparation and approval.

References

1. Barak M and Katz Y. Microbubbles: pathophysiology and clinical implications. *Chest* 2005; 128: 2918–2932.
2. Vann R, Butler F, Mitchell S, et al. Decompression illness. *Lancet* 2010; 377: 153–164.

3. Gerriets T, et al. Protecting the brain from gaseous and solid micro-emboli during coronary artery bypass grafting: a randomized controlled trial. *Eur Heart J* 2010; 31: 360–368.
4. Pugsley W, et al. The impact of microemboli during cardiopulmonary bypass on neuropsychological functioning. *Stroke* 1994; 25: 1393–1399.
5. Barak M, Nakhoul F and Katz Y. Pathophysiology and clinical implications of microbubbles during hemodialysis. *Semin Dial* 2008; 21: 232–238.
6. Neuman TS. Arterial gas embolism and decompression sickness. *News Physiol Sci* 2002; 17: 77–81.
7. Hagen PT, Scholz DG and Edwards WD. Incidence and size of patent foramen ovale during the first 10 decades of life: an autopsy study of 965 normal hearts. *Mayo Clin Proc* 1984; 59: 17–20.
8. Wilmshurst PT, Byrne JC and Webb-Peploe MM. Relation between interatrial shunts and decompression sickness in divers. *Lancet* 1989; 2: 1302–1306.
9. Moon RE, Camporesi EM and Kisslo JA. Patent foramen ovale and decompression sickness in divers. *Lancet* 1989; 1: 513–514.
10. Germonpre P, et al. Patent foramen ovale and decompression sickness in sports divers. *J Appl Physiol* 1998; 84: 1622–1626.
11. Cantais E, et al. Right-to-left shunt and risk of decompression illness with cochleovestibular and cerebral symptoms in divers: case control study in 101 consecutive dive accidents. *Crit Care Med* 2003; 31: 84–88.
12. Corretti MC, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; 39: 257–265.
13. Boyle EM Jr, et al. Microvascular responses to cardiopulmonary bypass. *J Cardiothorac Vasc Anesth* 1999; 13(4 Suppl 1): 30–35; discussion 36–37.
14. Verrier ED and Morgan EN. Endothelial response to cardiopulmonary bypass surgery. *Ann Thorac Surg* 1998; 66(5 Suppl): S17–S19; discussion S25–S28.
15. Miyazaki H, et al. Hemodialysis impairs endothelial function via oxidative stress: effects of vitamin E-coated dialyzer. *Circulation* 2000; 101: 1002–1006.
16. Vince RV, et al. Release of VCAM-1 associated endothelial microparticles following simulated SCUBA dives. *Eur J Appl Physiol* 2009; 105: 507–513.
17. Obad A, et al. Antioxidant pretreatment and reduced arterial endothelial dysfunction after diving. *Aviat Space Environ Med* 2007; 78: 1114–1120.
18. Obad A, et al. The effects of acute oral antioxidants on diving-induced alterations in human cardiovascular function. *J Physiol* 2007; 578(Pt 3): 859–870.
19. Madden LA, et al. Endothelial function and stress response after simulated dives to 18 msw breathing air or oxygen. *Aviat Space Environ Med* 2010; 81: 41–45.
20. Brubakk AO, et al. A single air dive reduces arterial endothelial function in man. *J Physiol* 2005; 566(Pt 3): 901–906.
21. Joannides R, et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* 1995; 91: 1314–1319.