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Endothelial microparticles in vascular disease and as a potential marker of decompression illness

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Key words
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Abstract

Micro-gas emboli are known to be present within the venous circulation following routine hyperbaric exposure. Emboli can be identified/quantified using Doppler and 2D ultrasound, thus functioning as an index of decompression stress. In relation to decompression illness this technique has low sensitivity and specificity. A biological marker of decompression stress would prove a useful tool. Such a marker could be used to gauge the efficacy of prophylaxis. Endothelial cells are known to shed microparticles during activation and apoptosis. Since microparticles in general express the antigens of the cells from which they were derived, the origin of them can be determined and their phenotype can lead to an insight as to the state of the parental tissue. Microparticles have been studied in many vascular diseases, and reviewed here, and we hypothesise that micro-gas emboli have a capacity to damage the endothelium and thus cause a change in the circulating microparticle population.

Decompression illness

When humans breathing air sojourn to high- or low-pressure environments they are exposed to the risk of acute decompression illness (DCI, the bends). The population at risk is varied and growing, e.g., sport scuba divers, submarine escapees and space explorers. The number of sports diving related cases of DCI has steadily risen in both North America and the UK over the last 10 years. Advancement in techniques for both prophylaxis and treatment of DCI is warranted.

Decompression illness is the result of gas phase forming in tissue and blood, following pressure changes, i.e., return to atmospheric pressure (surfacing) or a drop in pressure as in astronaut extravehicular activity. Modern technology has allowed the detection of venous gas following routine (believed) safe dive profiles. Contrary to the thinking 100 years ago, venous gas does not routinely lead to overt DCI. Venous bubbles often referred to as 'silent' appear to be filtered by the lungs and fail to reach the arterial circulation where their presence is significantly more problematic.

Historically decompression procedures (tables) have been validated following mathematical calculation, animal modelling and human trials. More recently, detection of venous gas, accepted as an index of decompression stress has been used to help validate decompression procedures, as has probabilistic modelling.

Vascular gas can be identified using Doppler and 2D imaging; however, these techniques have limitations. For example they tend to use short time weighted sampling (typically 5 minutes) over increasingly extended periods post exposure; they poorly quantify vascular gas. Their sensitivity and specificity in relation to DCI is poorly defined. There are numerous reports of divers with no or low bubble scores becoming ill and asymptomatic divers with high bubble scores.

With the now wide acknowledgement that DCI often attacks the central nervous system, human trials with DCI as one possible endpoint have become ethically and practically problematic. Finally the postulate that venous gas bubbles are 'silent' or biologically inactive regardless of their quantity is naïve. Accordingly a method of biologically describing a subclincial dose response to the effect of a decompression procedure would be helpful in all aspects of decompression modelling. A potential biological marker relating to the stress of a dive is the endothelial microparticle. Gas bubbles released into the circulation will interact with the surface of the vascular endothelium and may give rise to a measurable response, linked to the state of the endothelium.

Endothelial function

Vascular endothelium plays a role in the mechanisms of haemostasis, being involved with the vessel itself, platelet interaction and the functions of the plasma. Endothelial function also is implicitly involved in inflammation and repair of damaged tissue. Disruption of the endothelium, either physically or characteristically, due to a disease state such as post-ischaemic reperfusion, inflammation, hypertension and others, results in a potentially prothrombotic endothelial function. In this state the endothelium expresses von Willebrand factor (vWF), P-selectin (CD62P), ICAM-1 (CD54), VCAM-1 (CD106), IL-8 and promotes the activation and adhesion of platelets, via PECAM-1 (CD31).

Thrombin formation is also observed, along with expression of tissue factor and fibrin deposition. Vascular permeability increases and the production of cytokines, chemokines, and growth factors and the expression of cellular adhesion molecules are upregulated. These responses are usually part of endothelial function rather than dysfunction and are a prerequisite for tissue repair and wound healing subsequent to the disruption.

Endothelial dysfunction usually results from various disease states and is characterised by a reduction in dilatory capacity and decreased NO capacity, as a result of increased oxygen radical production, which reacts with NO to form peroxynitrate. NO and prostacycline (PGI_2) are vasodilators, involved in maintaining an antithrombotic state by preventing the formation of platelet aggregates. NO controls endothelium-dependent vasodilation, leucocyte adhesion, platelet aggregation, expression of adhesion molecules, synthesis of endothelin and inhibition of vascular growth and inflammation.[1] NO is produced in endothelium by nitric oxide synthase (eNOS) and is inactivated by oxygen radicals. Production of NO is dependent upon the presence of cofactors (such as tetrahydrobiopterin) and the availability of the substrate L-arginine. Oxygen radicals can be produced by eNOS under conditions of tetrahydrobiopterin or L-arginine deficiency and elevated concentrations of LDL-cholesterol. NADH/NADPH oxidase also produces oxygen radicals when stimulated by TNFα, and is located in the arterial wall, where extracellular superoxide dismutase acts to remove such radicals.

Endothelial dysfunction can be measured and is implicated in arteriosclerosis,[2] in which oxygen radical formation is enhanced.

Oxidative stress results in endothelium-mediated vessel dilation and a subsequent increase in cell turnover and death. Endothelial dysfunction is normally reversible.[3]

Cytokine activation of endothelial cells results in increased ability to bind circulating leucocytes, by as much as 400%.[4] This increase is due to new or increased expression of adhesion molecules E-selectin (CD62E), ICAM-1 and VCAM-1. Under normal physiological conditions endothelial cells bind leucocytes only briefly, but once activated low affinity interactions are formed, which are then disrupted by shear forces, to be reformed once again, causing a rolling of the leucocytes on the cell surface.

This in turn, with the involvement of chemokines, causes firm attachment of leucocytes to the endothelium, where they crawl to the endothelial cellular junctions and extravasate into the tissue space causing inflammation. Adhesion molecule expression follows a defined path; E-Selectin expression occurs early in the process of inflammation (around 2–4 hours after activation) and VCAM-1 expression later (12–24 hr). The pattern of expression can be modified by various chemokines, such as INFγ and IL-4.[5,6] E-Selectin, ICAM-1 and VCAM-1 possess DNA sequences that bind transcription factors NfκB and activator protein-1 and these are essential for the TNFα-mediated activation of endothelial cells,[7] showing that these transcription factors are able to modulate the adhesion molecule expression.

Endothelial microparticles and markers of endothelial perturbation

Established markers of endothelial cell (EC) damage/activation are traditionally soluble, and are measured from circulating blood. Such markers include ICAM-1 and VCAM-1 amongst many others. However, measurement of these markers may well include membrane-bound forms, as they can be removed by filtration.[8] This has led to a wide variation in measurements determined by ELISA techniques. These membrane-bound markers are constituents of microparticles (MP). Endothelium-derived MP (EMP) and the markers they express are indicative of the state of the endothelium, i.e., activated or apoptotic. They were first described as being released from cultured human umbilical vein endothelial cells (HUVECS) upon stimulation with complement,[9] and have been studied as an *in vitro* model for release of MP during activation or apoptosis.[10] MP released from HUVECS are phenotypically distinct and have been proposed to be a useful marker for endothelial injury,[11] and are presumed to be procoagulant due to the expression of anionic phospholipids.

Microparticles in humans

MP are released by unstimulated endothelium in healthy subjects, and so a basal concentration exists in the circulation. This suggests that endothelial vesiculation occurs under normal physiological conditions.[12] They have been postulated to maintain a balance between cell activation, proliferation and death and be involved in the maintenance of homeostasis.[13] Plasma membrane vesiculation is part of remodelling and there is evidence that MP can illicit a response in remote cells via their expressed antigens.[13] An increase above the basal levels of MP may lead to pathologic disorders; however, basal levels are not detrimental. As MP numbers vary according to the method used no comparable intra-study is available, although research to date has been compared to levels found in healthy controls under the same detection conditions.

Microparticles in disease

MP in the blood circulation have been described in many disease states as either increased in their numbers or being of altered composition, reviewed by Horstmann et al.[8] They were first identified from platelets by Wolf,[14] and have been shown to be released by many different cells in response to activation or cell death, recently reviewed by Nieuwland and colleagues in relation to their role in cardiovascular disease.[15] Release of MP from activated cells is time and calcium dependent,[16] whereas those released from cells

undergoing apoptosis are formed by membrane blebbing, and are positive for annexin V binding. In both cases MP carry the proteins specific to the parent cell from which they were derived, thus allowing identification of relative MP populations. This is particularly useful when the parent cell may have become activated and expressed proteins specific to the activated state.

Increased numbers of circulating MP have been studied in acute coronary syndromes,[17] multiple sclerosis,[18] arteriosclerosis,[15] diabetes,[19,20] hypertension,[21,22] pre-eclamplsia,[23,24] and sepsis,[25] amongst others. MP have been shown to be either elevated or of an altered composition in patients with cardiovascular disease that show impaired endothelial function.[15] MP released from endothelial cells may act as a marker for vessel wall injury.[10]

Microparticles in thrombocytopenic purpura

EMP, released from perturbed endothelium, were elevated in thrombotic thrombocytopenic purpura (TTP),[11] a disease where platelet activation is established. Plasma from TTP patients was found to induce a three-fold increase in ICAM-1 and a 13-fold increase in VCAM-1 expression on *in vitro* culture of renal microvascular endothelial cells (MVECS). EMP were elevated in patients with TTP, but not when the disease was in remission and therefore were stated to have the potential to be a useful marker of endothelial injury. CD62E and CD54 expression on EMP from TTP patients was found to be increased significantly[26] and of CD62E-positive EMP, 55% displayed expression on vWF. The authors concluded that the EMP were released from activated endothelium in TTP patients. EMP counts returned to normal upon remission. EMP were analysed from cultured brain and MVECS. CD31 and CD42b were used to identify MP of endothelial origin. EMP were found to be pro-coagulant when the cells were stimulated with TNFα (activation) or mitomycin C (apoptosis).[11]

Research into EMP markers by the same group showed that they possess different proteins, which were determined by whether the MP were formed by activation or apoptosis pathways in the endothelial cell of origin.[27] The expression of the inducible markers CD54, CD62E and CD106 was found to be increased in MP from activated cells, compared with those from apoptotic cells and control samples. Annexin V binding to MP was found to be increased in both activation and apoptosis.

Microparticles in coronary disease

EMP were found able to bind platelets *in vitro*, forming aggregates (EMP-P) with a potential involvement in thrombus formation.[28] MP were isolated from HUVECS by ultracentrifugation and incubated with isolated platelets before being labelled with CD105 and CD41a. Flow cytometry confirmed aggregates expressing both the endothelial (CD105) and platelet (CD41a) markers had been formed. Similar aggregates could be isolated from healthy subjects and almost all of those which were CD105 positive were also found to express CD31 and two markers of endothelial activation (MCP-1 and CD62E). Patients with stable coronary disease were found to have a significantly higher concentration of EMP-P (16.7 per μL whole blood) than healthy controls (7.1 per μL). A significant decrease in EMP-P concentration was observed during acute myocardial infarction, which was hypothesised to be due to involvement of these aggregates in thrombus formation in the infarct-related vessel. Levels of circulating EMP-P returned to pre-event concentration at 48 hr. A previous study observed an increase in EMP within blood of subjects with acute myocardial infarction;[17] however, the MP were higher measured days after onset and when compared to healthy controls.

It has also been demonstrated that MP isolated from patients with myocardial infarction have the potential to cause further endothelial dysfunction.[29] Rat aortic rings were incubated with MP isolated by ultracentrifugation from patients with myocardial infarction. It was concluded that these MP caused a high degree of endothelial dysfunction in healthy vessels by affecting the NO transduction pathway. The MP significantly decreased relaxations in response to acetylcholine in the aortic rings, and this observation was eliminated upon endothelium removal or the addition of a NO synthase inhibitor. The actual MP, if indeed there was a particular type responsible, were not analysed as to their cellular origin.

Microparticles in multiple sclerosis

The presence of CD31 on endothelium is a prerequisite for extravasation of leucocytes,[30] and was found to be increased in the serum of MS patients where brain gadolinium-enhancing lesions were present.[31] Circulating EMP were analysed for CD31 and CD51 expression in MS patients and were found to be elevated in disease exacerbation but not when the disease was in remission. The amounts of EMP were found to be 2.45, 0.58 and 0.86 x 10^6 per mL in MS exacerbation, remission and normal controls, respectively. The median value for all collected EMP was surpassed by 93% of patients with MS in exacerbation and 90% were below the median when in remission, suggesting strong evidence for a role of endothelial damage in the disease process.

Microparticles in sickle cell anaemia

Circulating EC have been analysed in sickle cell anaemia, a disease in which the vascular endothelium has a role in pathogenesis.[32] A correlation was established between acute painful episodes and circulating EC. Also, the circulating EC were found to express CD54, CD106, CD62E+P, suggesting the endothelium is in an activated state in the illness. MP

activation following hyperbaric exposure, as indicated by the increase in CD106. Further investigations are necessary to correlate changes in MP population with diving stress.

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Monoplace hyperbaric chamber use of US Navy Table 6: a 20-year experience [Abstract]

Weaver LK

Abstract

We report a 20-year experience at LDS Hospital, Salt Lake City, UT, using the US Navy Treatment Table 6 (TT6) in an oxygen-filled monoplace hyperbaric chamber (1985–2004). Air breathing was provided via a demand regulator fitted with a SCUBA mouthpiece while the patient wore a nose clip. Intubated patients were mechanically ventilated with a Sechrist 500A ventilator, with a modified circuit providing air, when specified. We treated 90 patients: 72 divers (decompression sickness (DCS) = 67, arterial gas embolism (AGE) = 5), 10 hospital-associated AGE, and 8 miscellaneous conditions. They received a total of 118 TT6 (9 TT6 in intubated patients). Ninety-four per cent of the TT6 schedules were tolerated and completed. The intolerance rate from two surveyed multiplace chambers was zero and 3% of 100 TT6 schedules each. Failure to complete the TT6 was due to oxygen toxicity (4) and claustrophobia (3). The US Navy TT6 was well tolerated by patients with DCS or AGE treated in monoplace hyperbaric chambers, but tolerance may not be as high as when treated in the multiplace chamber.

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