Gas bubbles may not be the underlying cause of decompression illness – The at-depth endothelial dysfunction hypothesis

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SUMMARY

Gas formed in tissues and the circulating blood due to decompression is thought to be a significant factor in the progression of decompression illness (DCI). DCI is a potential problem for a growing population of professional and recreational divers. We hypothesise that these gas bubbles are not the causative agent in progression of DCI, rather an exacerbating factor. Endothelial dysfunction caused by a temporary loss of haemostasis due to increased total oxidant status is postulated to be the cause in this at-depth endothelial dysfunction hypothesis. Breathing oxygen at any pressure increases the oxidant status in the circulation causing vasoconstriction; this increase can be prevented by antioxidants, such as Vitamin C, maintaining haemostasis and preventing activation of endothelium, leukocyte recruitment and subsequent localised inflammation. Bubbles have the potential to exacerbate the situation on decompression by damaging the vascular endothelium either through ischemia/reperfusion, physical contact with the endothelium or by an increase in shear stress. Furthermore, this damage may manifest itself in the release of endothelial membrane fragments (microparticles).

Introduction: bubbles and decompression

During a dive, compressed air is taken up and saturates the surrounding tissues. During decompression to the surface, some of this gas is released from the tissue in the form of bubbles. It is commonly accepted that it is the formation of bubbles and their effects to the body that are the main causative effector of decompression illness (DCI). DCI occurs prominently in relation to compressed air or mixed gas diving or following rapid ascent to altitude. This can result in symptoms covering a range from joint pain to cerebral arterial gas embolism. Predicting the occurrence DCI is almost impossible when appropriate decompression schedules have been followed but it does happen. Bubbles can be detected using ultrasound Doppler imaging but the sensitivity and specificity to decompression illness is poorly defined. Divers with no or low bubble score have been shown with symptoms of DCI and divers with high bubble scores without symptoms [1].

The established view is that gas bubbles formed upon decompression have the potential to occlude vessels, causing ischemic events followed by reperfusion injury in the microvasculature. Also there is evidence that bubbles can interaction with the endothelium causing mechanical damage, including cell-stripping and an increase in the size of the cell junctions between the endothelial cells [2]. Blood viscosity may be increased due to the presence of

bubbles and complement is activated [3]. Circulating proteins can adsorb to the surface of bubbles through simple hydrophobic interactions, forming a protein layer which results in unfolding of the tertiary structure and ultimately makes possible biological interaction of the bubble with the endothelium [4]. This results generally in an inflammatory pro-thrombotic state and symptoms of DCI can present long after decompression.

The at-depth endothelial dysfunction hypothesis

Gas bubbles alone are not enough to cause decompression illness, they merely exasperate the situation upon decompression; rather it is a sickness related to transient loss of endothelial haemostasis caused by hyperoxia-induced vasoconstriction at depth and can therefore be protected against by endothelial preconditioning. This may take the form of exercise preconditioning or dietary supplementation with antioxidants.

Markers of DCI

Gas bubbles released into the circulation, following decompression are a poor predictor of the likelihood of symptoms [1]. Serum markers have been studied in their relation to DCI [5], however results are mixed and no marker has been found relating specifically to DCI. If our hypothesis is true then no freely soluble serum marker will be found for DCI, as symptoms of DCI appear linked to endothelial state and thus we proposed membrane fragments released due

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to vascular remodelling at depth and possibly upon decompression as a possible predictive marker of susceptibility to DCI [6]. The advantage of MP phenotyping is that the markers present on these membrane fragments were expressed on the cell of origin at the time of release and therefore MP are a useful tool to assess the state of the endothelium *in vivo.*

Importantly, hyperbaric oxygen (HBO) has been shown to attenuate the tethering of neutrophils to the endothelium possibly via a reduction in CD18 expression [7]. It seems that the endothelium must be involved in DCI as neutropenic rats appeared incapable of showing symptoms of DCI [8]; leading to suggestions that adhesion molecules upregulated by the endothelium, may be involved in DCI [5]. HBO inhibits neutrophil adherence [9] and platelet activation has been observed post-decompression which was found to be reduced with oxygen pre-treatment before a dive [10]. Any protective mechanism must therefore focus not on bubble formation but blood biochemistry and endothelial conditioning. Whatever form this may take, if cell attachment to the endothelium can be attenuated then we may be some way to preventing DCI, at least the less severe type I DCI, where symptoms appear inflammatory-related.

If gas bubbles were solely responsible for the symptoms of DCI then it should be regarded as unsafe to conduct human trials where the specific aim is to introduce gas bubbles into the circulation, however, the standard test for PFO involves directly introducing agitated saline into the venous system [11] In any case we highlight other factors that may be implicitly involved in the progression of DCI.

Endothelial activation

Endothelial function is impaired following a dive [12] and this observation is implicit to the at-depth endothelial dysfunction hypothesis. The vascular endothelium plays a major role in the whole body, being involved in inflammation and repair of damaged tissue. Disruption to the endothelium causing damage or cellular activation, results in a generally prothrombotic state. In a state of endothelial activation there is an induction of adhesion molecule expression. These markers are known to be expressed at different times following endothelial stress or insult. For example, P-selectin can be activated within minutes to support the cell adhesion process, whereas E-selectin peaks ∼4–6 h after activation. Increased expression of ICAM-1 and VCAM-1 follows and can stay upregulated for days [13]. VCAM-1 and its ligand VLA4 are implicitly involved in localised inflammation, responsible for leukocyte adhesion, transmigration, homing and activation [14]. As DCI manifests symptoms similar to anaphylaxis [5], it is possible that cytokine-mediated endothelial activation follows a dive and results in endothelial dysfunction. It is known that endothelial activation can be attenuated by vascular preconditioning, induced by either exercise or diet, resulting in decreased expression of activation molecules and thus maintenance of haemostasis.

Oxidative stress and the endothelium

Breathing oxygen at higher than normal (hyperbaric) pressures leads to an increased oxygen tension within the general circulation [15]. This in turn has the potential to cause biochemical changes and disrupt cellular haemostasis. This effect has been utilised as a protective agent before, for example, cardiac surgery [7,16]. High arterial oxygen tension, caused by breathing air at pressure or oxygen (normobaric and hyperbaric), increases vascular resistance, leading to shift away from haemostasis but is generally accepted to be a safe procedure. Therefore during a dive at depth there is an increase in vascular resistance due to vasoconstriction, which

may be linked to hyperoxia-dependent release of superoxide anions, thereby reducing bioavailability of nitric oxide (NO), which controls vascular tone.

Preconditioning

To focus on oxidant status and its effect on the endothelium; gas bubbles may form at the endothelial surface where conditions are thought favourable through mathematical modelling [17]. If in some way the endothelial surface could be modified to make a less favourable environment then bubbles may not form, or at least may be smaller. The endothelium is highly sensitive to oxidative stress; whether it is possible decrease this stress response via preconditioning appears an interesting proposition. Indeed exercise preconditioning in athletic training is very well established, as is the live high, train low philosophy. Furthermore, exercise preconditioning has been shown to prevent DCI in rats [18]. Modification of the endothelial response may be achieved simply through diet, for example a low fat diet high in antioxidants, this may also be achieved through exercise preconditioning [19] which elicits a stress response through release of antioxidants and heat shock protein induction. Since most recreational divers may be considered relatively fit, those which contract DCI must have a transient period where they are considered at risk, possibly a temporarily high oxidant status. Whether this could be attributable to something as simple as diet is a matter of speculation.

Antioxidant preconditioning

Endothelial preconditioning, improving antioxidant capacity, could be beneficial in the form of diet preconditioning, for example, through administration of supplements capable of reducing oxidant status, such as Vitamin C. Vitamin C has been demonstrated as a superoxide scavenger capable of reversing endothelial dysfunction caused by oxidative stress [20], suggesting that hyperoxic vasoconstriction is mediated by oxidative stress and these effects, countered by Vitamin C, show that superoxide impairs endothelial function. Merely breathing gas mixtures at increased pressures, such as diving at depth, therefore could account for the observed impairment of endothelial function post-decompression [12], via the above mechanism. This, coupled with the presence of gas bubbles in the circulation could exasperate DCI in the at-depth endothelial dysfunction hypothesis. Furthermore, supplementation with Vitamin C [21], thus increasing bioavailability of NO could confer some protection to this hyperoxia related constriction and negate any possible effect of subsequent gas bubble formation upon decompression.

Hyperoxia has been demonstrated to result in a significant (28% +/− 10) increase in forearm vascular resistance in healthy subjects [20]. Furthermore administering Vitamin C before hyperoxic breathing prevented this change (2% +/− 3). The authors concluded their data was consistent with hyperoxia impairment of endothelial function via a free radical mechanism, which could be prevented via administering Vitamin C.

Could a simple Vitamin C supplement be enough to confer endothelial protection via an oxidative stress mechanism? Recently we conducted an assessment on healthy males and found a link in two independent studies, between oxidant status (as measured by serum thiobarbituric acid reactive substances) and endothelial (VCAM-1 positive) MP release from activated endothelium [22]. If, as we have proposed, MP have the potential to be reflective not only of decompression stress but the state of the endothelium *in vivo*, then we have a measure by which oxidative stress preconditioning may be measured. Obviously this research is at an early

stage but the potential is established in MP research in patients with disease linked to inflammation. Furthermore, Vitamin C supplementation has already been shown to reduce both platelet and endothelial MP release in 61 patients with myocardial infarction [23], thus linking oxidant status and endothelial MP release in agreement with our own study on healthy volunteers.

Indeed the endothelium has been the subject of recent research on DCI. Obad et al. [21] have observed a decrease in flow-mediated dilation (FMD) post-dive, probably due to increased vascular resistance in response to increased oxygen tension, as previously discussed. This effect was countered somewhat by Vitamin C supplementation, however Vitamin C was administered orally in this study which could account for the partial restoration of FMD, rather than complete restoration seen in other studies using intravenous injection, suggesting a dose-dependant response, as may be expected.

Vitamin C improves endothelial function

Oxidative stress accounts for a high degree of endothelium dysfunction in atherogenesis [23] and antioxidant status has been shown to be correlated to clinical presentation of coronary artery disease [24]. Furthermore, low Vitamin C levels were linked with inflammation and disease severity [25]. Vitamin C supplementation has been demonstrated to improve endothelial function in patients with coronary artery disease [26,27]. Diabetic patients presenting with myocardial infarction were shown to have a reduction of up to 70% in circulating endothelial MP after treatment with Vitamin C [23]. The authors concluded that antioxidant treatment may improve endothelial function where disease is linked to high oxidative stress. Impaired endothelium-dependent vasodilation (EDV) is seen even at early stages of atherosclerosis and is associated with cardiovascular risk factors such as hypertension and diabetes mellitus (DM) [28]. Vitamin C is an effective scavenger of superoxide anions which can lead to an increased availability of NO, thereby improving EDV. Vitamin C has been shown to modify EDV in patients with hypertension, DM and also after high fat meals [28]. The authors demonstrated a protective effect of Vitamin C against elevated non-esterified fatty acids by scavenging superoxide anions. Endothelium-dependent vasodilation in response to acetylcholine is blunted in hypertensive patients and was seen to markedly improved by Vitamin C [29], furthermore this response was not seen in healthy subjects suggesting under normal physiological conditions Vitamin C does not have an effect due to the lack of oxidative stress. If, when diving, increases in oxygen tension blunt the vascular response this then would be analogous to temporary hypertension that should respond to Vitamin C via free radical scavenging.

Testing the hypothesis

Making firm conclusions taking small animal data into a human model is fraught with difficulty. To this end a human model of DCI would be ideal but obviously unethical as to fully test our hypothesis would ultimately lead to DCI. Furthermore experiments focussing on introducing bubbles into the venous system have been carried out over many years, mainly without any clear conclusions being able to be drawn from the data obtained. Blood samples would need to be taken at depth, before any decompression has played its part, and analysed accordingly.

Conclusions

The evidence presented here and elsewhere points to a definitive role of loss of endothelial haemostasis in the progression of DCI. Historically bubbles show no sensitivity or specificity in relation to DCI and therefore cannot be used as a prognostic or diagnostic marker. Breathing higher partial pressure of oxygen, when diving results in an increase in arterial oxygen tension, which in turn causes an oxidative stress related vasoconstriction. This can lead to increases in shear flow and vascular remodelling in the form of increased adhesion molecule expression and release of microparticles. These biological changes happen at depth and decompression, which leads to the formation of gas bubbles in the circulation and acts a secondary insult forming the basis of the at-depth endothelial dysfunction hypothesis. Prevention of endothelial dysfunction therefore could negate any effect of bubbles and symptoms of DCI.

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