THE VASCULAR ENDOTHELIUM CURRENT CONCEPTS OF CIRCULATORY HOMEOSTASIS AND PATHOPHYSIOLOGY.

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Key Words

Cardiovascular, physiology.

Abstract

The vascular endothelium is increasingly recognised as an active tissue, with key roles in the maintenance of a non-thrombogenic, semi-permeable circulatory barrier, variable vascular tone, and the regulation of platelet reactions and leucocyte trafficking. Central to these functions are the endothelial derived mediators of nitric oxide, prostanoids and the cell adhesion molecules.

Nitric oxide is produced by healthy endothelium and acts as a flow dependent vasodilator, an inhibitor of platelet aggregation and a regulator of CAM production. Prostacyclin, the principal endothelial prostanoid, is both vasodilatory and a potent anti-platelet agent. The cell adhesion molecules include selectins, integrins, and members of the immunoglobulin superfamily. They participate in crucial cell-cell and cell-matrix interactions such as platelet activation, leucocyte adhesion and migration of cells to sites of inflammation.

The dependence of a wide variety of disease states, from myocardial infarction to decompression illness, on these reactions opens up new therapeutic potential for intervention with novel agents.

ABBREVIATIONS TABLE

5HT	5hydroxytryptophan
AA	arachadonic acid
ACE	angiotensin converting enzyme
ACEI	ACE inhibitors
ADP	adenosine diphosphate
Ang II	angiotensin II
ATIII	anti-thrombin III
CAM	cell adhesion molecules
COX	cyclo-oxygenase
CPB	cardiopulmonary bypass
ECE	endothelin converting enzyme
EDHF	endothelium dependent hyperpolarising factor
EDRF	endothelium derived relaxing factor
eNOS	endothelial nitric oxide synthetase
ET-1	endothelin
IFNg	interferon gamma
IL1	interleukin 1
IL8	interleukin-8
NO	nitric oxide
NOS	nitric oxide synthetase
nNOS	neuronal nitric oxide synthetase
PG	prostaglandin
PGI_2	prostacyclin
SAH	subarachnoid haemorrhage
TF	tissue factor
TNF	tumour necrosis factor
TxA2	thromboxane
vWF	von Willebrand factor

Introduction

Over the last decade our understanding of the endothelium has evolved from that of a simple lining of the vascular tree into one of a specialised tissue with a range of complex functions. The interrelation of these often opposing functions at a molecular level is only now starting to be unravelled. This overview aims to highlight some of the recent advances in endothelial function that are particularly relevant to general and underwater medicine.

Vascular Tone

ENDOTHELIAL VASODILATORS

Vascular tone has traditionally been viewed as balance of direct acting, sympathetic and para-sympathetic, constrictive and dilatory effects on vascular smooth muscle. Local factors such as temperature, pH and PCO_2 regulate minute to minute blood flow to specific tissues. Furchgott was the first to demonstrate that vasodilators could have indirect actions, via the endothelium, on vascular tone.[1] It is now well recognised that the potent vasodilator, endothelium derived relaxing factor (EDRF) is responsible for the effects of many intrinsic and exogenous vasodilators (fig 1).

EDRF has subsequently been identified as the ultra-short lived product, nitric oxide (NO), and/or more persistent thiol-adducts (proteinaceous complexes of NO with sulphur containing amino acid residues). NO is produced from L-arginine through the action of the nitric oxide synthetase (NOS) family of enzymes. NO is released into the vessel lumen where it exerts anti-platelet effects, and into the vessel wall, producing vasodilatation. Nitric oxide synthetase is always present on healthy endothelial surfaces (eNOS) and possibly also in the bronchial epithelium. Its activity is increased by shear stress and by oestrogen. Many intrinsic (bradykinin, histamine, acetylcholine, serotonin) and extrinsic vasodilators (various drugs) act to increase eNOS activity and NO generation. Therapeutic nitrates, in the form of glyceryl trinitrate, and isosorbide mono- (Imdur) and di-nitrates (Isordil) are metabolised by the endothelium to release NO, whereas nitroprusside, a direct NO donor is an endothelium independent vasodilator. Both thrombin and endothelin

increase eNOS activity, which may serve to counteract the vasoconstrictive properties of these agents.

The first pathophysiological role ascribed to NO was in subarachnoid haemorrhage (SAH). Free intracranial haemoglobin is a potent inactivator of NO and contributes to the development of vasospasm in SAH. Volume expansion in this setting increases shear stress and so NO production. The diffuse vasospasm of SAH may be (in part) analogous to that seen in neurological decompression illness (DCI).

Large amounts of NO can be produced by inflammatory cells in response to stimulatory cytokines (eg tumour necrosis factor and interferon g). NO toxicity contributes to the adverse negative inotropism, peripheral vasodilatation and pulmonary congestion of septic shock. Indeed, persistent exposure of many cell types to high levels of NO (e.g. in septicaemia) leads to their apoptosis (programmed cell death).

A second potent endothelial derived vasodilator is prostacyclin (PGI_2), which is produced mainly in small arterioles and capillaries. Prostanoids are derived from arachadonic acid (AA), by the actions of cyclo-oxygenase (COX) and then specific prostaglandin (PG), prostacyclin and thromboxane (TxA_2) synthetases. PGI_2 may have only a minor vaso-active role in health but is an important anti-platelet agent. PGI_2 is increased in diabetics, and contributes to their higher resting limb blood flow (independent of any autonomic neuropathy) compared to non-diabetics. However in diabetes, complex endothelial dysfunction leads to an overall impairment of normal exercise induced vasodilatation, largely due to impaired NO production.

The third endothelial vasodilator was initially described on the basis of functional "endothelium dependant vaso-relaxation", not inhibited by a combination of NOS and COX blockers and which was associated with hyperpolarised arterial smooth muscle. This substance has been termed endothelium dependent hyperpolarising factor (EDHF) or endocannabinoid, as its vascular effects are mediated by binding to intrinsic cannabinoid receptors. EDHF contributes significantly to vasodilatation of intra-myocardial arteries, and generation of EDHF is thought to contribute, in part to the coronary dilation of bradykinin.

ENDOTHELIAL VASOCONSTRICTORS

The most potent vasoconstrictor identified to date is the polypeptide endothelin (ET-1). ET-1 is formed from the sequential enzymatic cleavage of a larger precursor protein. The final step is catalysed by endothelin converting enzyme (ECE), in a manner analogous to the angiotensin converting enzyme (ACE) dependent synthesis of angiotensin II (Ang II) (fig 1). Adrenalin, Ang II, chronic hypoxia (e.g. high altitude), and thrombin increase the

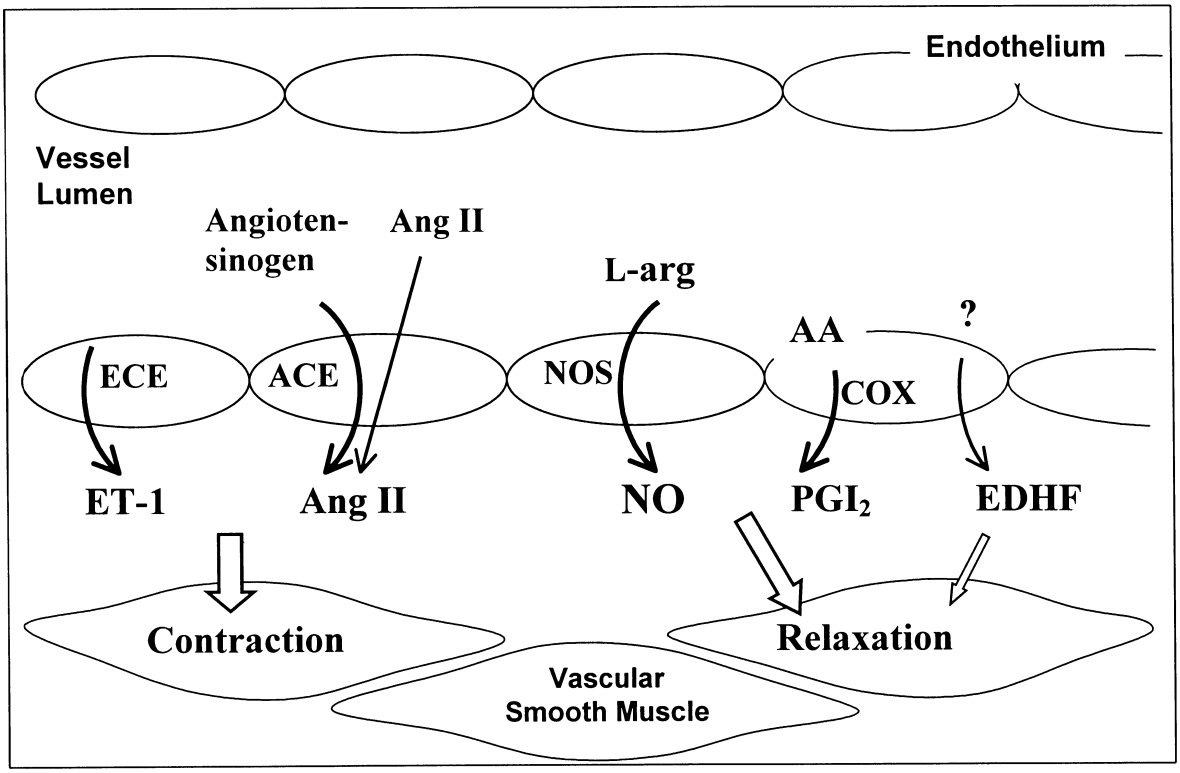

Figure 1. Maintenance of vascular tone by a balance of vasodilating and vasoconstricting substances. For abbreviations, refer to abbreviations table.

production of ECE and hence ET-1 production. Circulatory levels of ET-1 are very low, suggesting it acts mainly locally within a few mm of where it is produced. Its vasoconstriction is more potent on veins, intramyocardial-coronary and pulmonary arteries. ET-1 acts on ET_A receptors on vascular smooth muscle to produce vasoconstriction and on endothelial ET_B receptors leading to NO and PGI_2 production. The first clinically relevant ET receptor antagonist, bosentan, is undergoing phase III trials for the treatment of heart failure and primary pulmonary hypertension.[2]

The renin-angiotensin system is an important regulator of both vascular tone and Na+/K+ balance. Local tissue ACE and Ang II generation is known to be important in regulating regional blood flow and angiogenesis, as well as in myocardial remodelling after infarction. Ang II vasoconstricts via a direct action on smooth muscle angiotensin receptors and by increasing local ET-1 production. ACE inhibitors (ACEI) causes vasodilatation by inhibiting these mechanisms, by enhancing renal Na+ excretion, and by reducing the breakdown of bradykinin. The benefits of ACEI are limited alternative mechanisms of Ang II generation, involving tissue chymases and other enzymes. This has lead to the development of specific AT_1 receptor antagonists, including irbesartan (AvaproÆ), which has been released in the Australasian market place. It is likely that this new therapeutic class will have synergistic effects with ACEI.

The vasoconstricting effects of the prostanoids TxA_2 and prostaglandin H2 (PGH_2) are relevant to local responses to endothelial damage and platelet plug formation. They are otherwise not considered to play a major role in homeostasis of vascular tone.

Haemostasis : Platelets and Thrombosis

Normal haemostasis is a complex interplay between the vessel wall, platelets and the coagulation and fibrinolytic systems.

In the absence of vascular disruption a non-thrombogenic, semi-permeable, endothelial barrier must be maintained. This is achieved in part by high local concentrations of PGI_2 and NO to which platelets are exposed during their capillary transit. The anti-platelet effect is sufficient to ensure that most platelets remain inactivated under normal conditions. In all but the smallest blood vessels, normal laminar flow prevents the formed elements of blood from coming into contact with the endothelium. Additionally, intact endothelium produces thrombomodulin, heparan sulphates (Heparans are a similar but distinct group of native glycose-amino-glycan substances to the broad group of substances known as therapeutic heparin.) and anti-thrombin III (ATIII) proteins, which inactivate thrombin and stimulate the fibrinolytic system. The endothelium is also a potent source of ADPase, the enzyme that breaks down adenosine diphosphate (ADP) which is produced by platelets.

After endothelial damage, blood elements are exposed to collagens, tissue factor (TF) and von Willebrand factor (vWF) in the subendothelial matrix. An initial platelet monolayer is formed in response to adhesion of their surface glycoprotein-Ib receptors to tissue vWF. Platelet degranulation (the "release reaction") ensues with 5hydroxytryptophan (5HT) and ADP excretion leading to further recruitment of platelets. The final step of platelet activation is the production of glycoprotein-IIb/IIIa, which allow fibrinogen binding and further platelet accumulation. The multilayered "platelet plug" so formed provides initial haemostasis (Fig 2). Subsequent "fluid phase" haemostasis is dependent on activation of the coagulation system. This is enhanced by the production of TF on the surface of activated platelets and endothelial cells. TF production is increased by hypoxia and by inflammatory cytokines such as interferon gamma (IFNg) interleukin 1 (IL1) and tumour necrosis factor (TNF). Thrombin is generated by the coagulation factor Xa and leads to fibrin cross linking (with stabilisation of the platelet plug), further platelet activation, release of various vasoactive endothelial substances and stimulation of fibrinolytic pathways.

The events contributing to thrombosis are endothelial damage with platelet activation, stimulation of the coagulation cascade, reduced local PGI_2 and NO formation producing vasospasm, loss of laminar flow, stasis and further accumulation of activated platelets and coagulation factors.

Leucocyte trafficking and migration

Trafficking is the term used for the two-way movement of leucocytes between the circulation and tissues, both during normal cell recirculation, and during migration into areas of tissue injury or inflammation. The ability of leucocytes to be rapidly recruited to areas of tissue injury is dependent on the variable production and activation of specific cell adhesion molecules (CAM) on both the endothelium and leucocytes. The three major families of CAM involved in regulation of leucocyte migration are the selectins, the integrins and the immunoglobulin superfamily (Table 1). These facilitate the sequence of leucocyte rolling, activation, tight adherence and tissue migration (fig 3, page 236).

Endothelial cells have low levels of both E- and P-selectin on their luminal surface which are rapidly increased in response to local tissue injury. Endothelial selectins interact reversibly with L-selectin on the surface of leucocytes, slowing leucocyte flow in a process termed "rolling". This facilitates subsequent leucocyte activation by local cytokines and enables cell-cell interactions that

would otherwise be prevented by normal vascular shear rates.

The integrins consists of a series of a and b-chain heterodimers, which are sub-classified according to the b-chain. Leucocyte migration principally involves the b_1 (CD29) and b_2 (CD 18) families. On leucocytes, surface production of CD 11/18 is markedly increased when cells are activated by complement, leukotriene B4, TNF or the interleukin-8 (IL8) family of chemokines. The b_3 integrin, gp IIb/IIIa, forms the principal adhesion molecule of activated platelets.

The adhesion molecules ICAM (1,2 & 3), VCAM and PECAM (CD 31) belong to the immunoglobulin superfamily. Endothelial ICAM and VCAM are the counter receptors for b_2 and b_1 integrins respectively. Normal endothelial ICAM production is low, but increases rapidly upon endothelial activation. After the initial selectin mediated rolling phase, the formation of ICAM-integrin pairs leads to tight adherence by activated leucocytes and allows subsequent migration.

Endothelial Dysfunction

Endothelial dysfunction was initially recognised to occur in patients with established atherosclerosis. Elegant cardiac angiographic studies showed paradoxical

TABLE 1

CELL ADHESION MOLECULES

Major CAM Families

Selectins			
	L-selectin	P-selectin	E- selectin
Integrins			
	b1	VLA-4	
	b2	CD 11a/18	11b/18 11c/18
	b3	IIb/IIIa	Vibronectin receptor
Immunoglobulin superfamily			
	ICAM-1,2	VCAM	PECAM-1

Leukocyte Migration and CAM Pairs

Rolling		
L selectin	-	P & E-selectin
Triggering		
CD31	-	chemokines, integrins
Adhesion		
CD 11b/18	-	ICAM
VLA-4		VCAM
Migration		
CD31	-	extracellular mucopolysaccharides

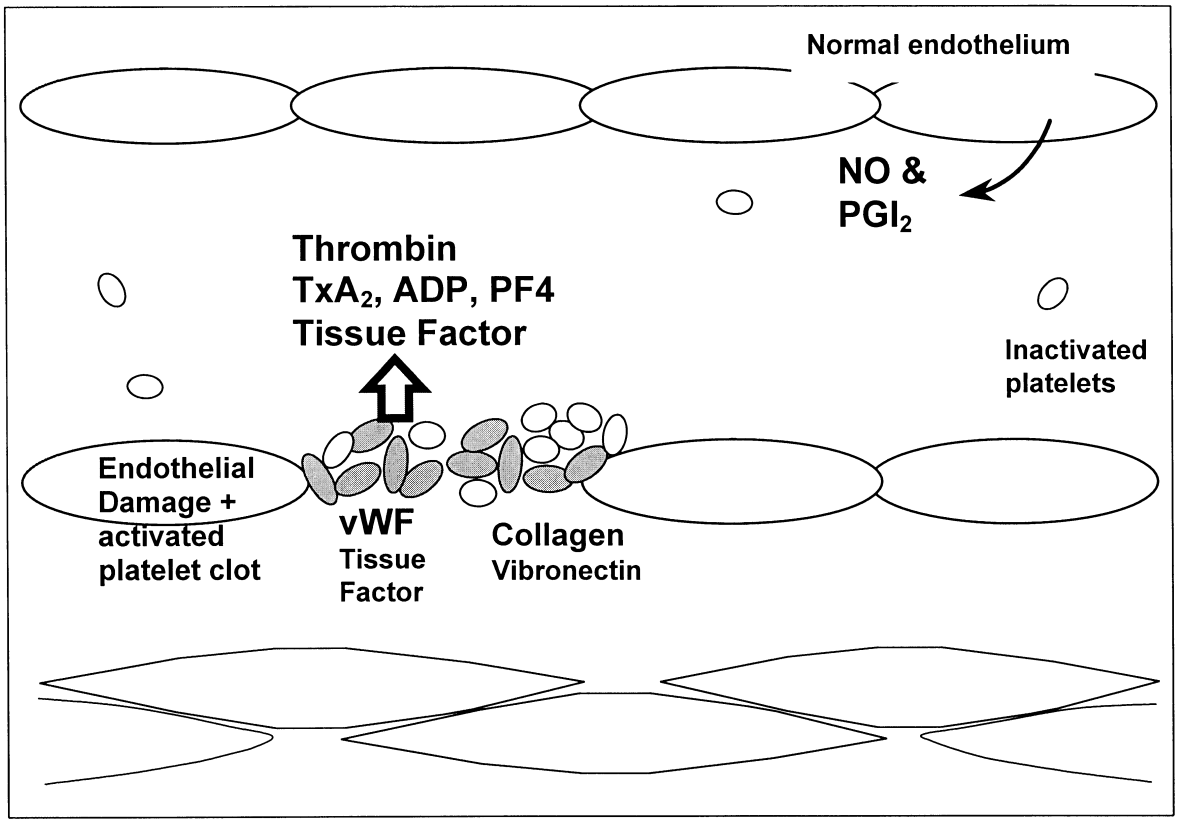

Figure 2. Formation of a platelet clot in response to endothelial injury.

vasoconstrictive responses to acetylcholine and serotonin. This was followed by the demonstration of exercise induced vasospasm, a contrast to the normal flow mediated vasodilatory response. It is now recognised that these phenomena are largely a reflection of impaired NO (and/or PGI_2) production by the endothelium at sites of atherosclerosis. Endothelial dysfunction can be measured by a number of non-invasive means, particularly by the use of flow mediated vasodilatation. These functional changes are seen in a number of conditions traditionally associated with a risk of subsequent vascular disease (Table 2). Endothelial dysfunction is now thought to be a marker for the development of atherosclerosis.

Impaired NO production is also associated with an increase in endothelial CAM production and hence leucocyte adhesiveness. Increasingly, inflammatory cells are recognised as playing an important role both in the development of atherosclerosis and in the conversion of stable atheroma into unstable disease. The changes in CAM production and in NO/PGI_2 release favour leucocyte accumulation. Lymphocyte actions within atheromatous plaque drive pro-coagulant pathways, such as macrophage TF production. When stimulated (e.g. by intercurrent infections) they release IFNg that is a major contributor to weakening of the plaque structure and increasing its propensity to plaque rupture.

Whether such endothelial dysfunction and/or atherosclerosis is a predisposition to other vascular disease states such as decompression illness can only be conjectured.

Reperfusion injury and cardiac bypass

Cardiopulmonary bypass (CPB) has long been recognised as causing a variety of acute vascular and haematological effects. These can be broadly separated into the effects of exposure of blood elements to the CPB circuitry and the effects of ischaemia-reperfusion in the coronary and pulmonary vascular beds.

Platelets bind to the synthetic materials of the by-pass circuit and this contributes to the mild fall in platelet count seen after CPB. Complement activation, with the generation of complement, leads to leucocyte activation, with subsequent generation of leukotriene B4 and increase in CD 11b/18 production.

During CPB, the ischaemic myocardial and pulmonary circulations increase P-selectin and ICAM production. Reperfusion of areas is associated with neutrophil sequestration and generation of activation products such as ROIs. In the lungs, increased endothelial permeability leads to the development of interstitial oedema and alveolar collapse, hypoxia and, in extreme cases, adult respiratory distress syndrome (ARDS). Similar reperfusion injury to the myocardium is associated with (transient) impairment of myocardial contraction (known to cardiologists as “stunning”).

There is increasing interest in the role of platelet-leucocyte interactions. Stimulated platelets can bind to neutrophils and cause their activation. In addition,

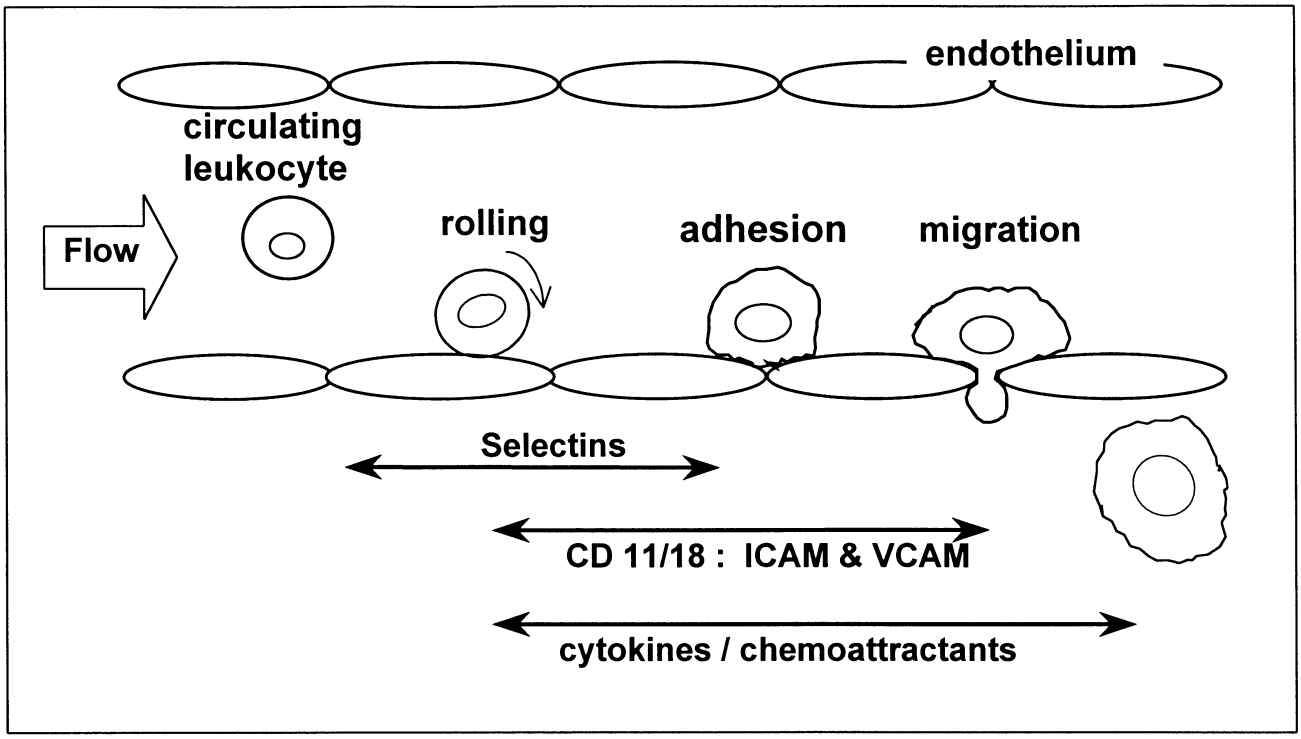

Figure 3. Schematic diagram of a leukocytes rolling on the endothelium, becoming activated by local cytokines and adhering to the endothelium, and then transmigrating out of the vascular space.

heparin, routinely given with CPB, causes platelet micro-aggregation. This effect is (partially) reversible by later protamine administration. Platelet micro-aggregates are readily demonstrable during CPB; they have the potential to cause microvascular obstruction and contribute to post-operative thrombocytopenia.

Doppler ultrasound has been used to identify and quantify vascular bubbles. The Doppler signal is thought to represent micro-bubbles from intravenous fluids, and during CPB, from "leak" of free gas from oxygenators. Both new cognitive neuropsychiatric deficits and MRI lesions after surgery correlate with the extent of Doppler signal intensity pre-CPB, raising the possibility that Doppler may also be detecting non-gaseous embolic material. At present there is no clear link between this observation and the phenomena of heparin-induced platelet aggregation or leucocyte/platelet interactions or activation.

Therapeutically, the administration of monoclonal antibodies against CD 11b/18 leads to a reduction in both neutrophil sequestration and hypoxia in animal models of CPB. In human observations, administration of an antibody to platelet gp IIb/IIIa (vide infra) has been associated with a reduction in post-CPB thrombocytopenia.

Decompression Illness

The nature of decompression illness has been partially elucidated in recent years. Our current understanding involves a bubble mediated endothelial injury that leads to neutrophil activation and adherence. The neutrophil response presumably occurs secondary to increased CAM and/or chemotactic cytokine production. Intra-cerebral vasospasm leads to the clinical syndrome of cerebral decompression sickness. The mechanism of neutrophil-induced vasospasm remains speculative. Neutrophil activation products such as ROIs are known to both inactivate NO and to inhibit NO synthesis by NOS. Additional vasospastic mechanisms are likely to be involved.

It is uncertain why some subjects form circulating bubbles after hyperbaric air exposure, and why despite bubbling being relatively common, only a small proportion of patients apparently suffer from these vasospastic phenomena. Also, the mechanism of benefit of hyperbaric oxygen therapy (HBO) remains speculative. Animal models of pulmonary bubble injury (air embolism) are associated with increased vascular permeability and this is exacerbated by neutrophil and complement activation. HBO can reduce neutrophil sequestration and activation in the rat gracilis muscle flap model of ischaemic-reperfusion, and can reduce b_2 integrin (CD 18) dependant binding of human neutrophils by a mechanism dependant on impairment of cGMP synthesis.

The potential benefits of HBO may be offset by the possibility of oxygen toxicity. HBO causes an oxidant stress to the lungs with increases in pulmonary neutrophil activation and ROI generation and with reduction of ROI catabolism (by phospholipase A_2). The neurological toxicity of oxygen is paradoxically associated with increased cerebral NO, and can be prevented by inhibitors of neuronal NOS or monoamine oxidase.

The mechanism of benefit with HBO may depend on the specific clinical circumstance in which it is being applied. There is increasing interest in the role of neutrophil antagonists to both reduce the vasospasm of DCI and prevent the toxicity of HBO.

New therapies

A variety of new therapies targeting adhesion molecules are currently being evaluated or entering clinical practice.

The first therapeutically useful anti-platelet agents are blockers of the gp IIb/IIIa receptor, which inhibit the final common pathway of platelet activation. ReoProÆ (generic name abciximab) is a chimeric antibody fragment; specific mouse anti-IIb/IIIa binding domains have been cloned onto human immunoglobulin constant regions to produce a minimally antigenic molecule. ReoProÆ given intravenously is a rapid acting, potent platelet antagonist that has proved useful in the treatment of complicated angioplasties. The next generation of specific, parenteral, non-protein IIb/IIIa blockers (e.g. tirofiban Æ produced by MSD) have completed phase 3 trials and may be available to the marketplace shortly, to be followed by orally active agents in the next 2-3 years.

Monoclonal antibodies have been shown to be useful in a variety of animal models of ischaemia-reperfusion. Similarly, soluble CAM fragments may prove useful as competitive antagonists of their counter receptors.

TABLE 2

ENDOTHELIAL DYSFUNCTION

Causes and Associations

Hypercholesterolaemia	Homocystine
Hyperglycaemia	Hyperinsulinaemia
Smoking	Oestrogen withdrawal ?
	Atheroma

Consequences

Reduced endothelial NO / PGI_2 production
paradoxical vasoconstrictive responses
Increased adhesion molecule expression
increased platelet & leukocyte adhesiveness
Susceptibility to vascular disease(s)

Soluble P-selectin has been used experimentally to bind to leucocyte L-selectin, thus preventing normal leucocyte rolling on activated endothelium. Antagonists of CAMs are likely to undergo a similar process of development as that of the IIb/IIIa blocking anti-platelet drugs.

Conclusions

The complex and interrelated functions of the endothelium in health are being slowly unravelled. With this our understanding of endothelial abnormalities in a variety of diverse disease states is increasing. In the near future we are likely to see major therapeutic advances based around the roles of these endothelial derived mediators.

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OCCUPATIONAL DIVERS' KNOWLEDGE

Professional diver knowledge of diving medical issues, a pilot study.

Strauss MB, Borer RC Jr and Borer KM. *Undersea Hyperbaric Med* 1998; 25 (Suppl): 41

Abstract

Background

Professional divers are those individuals who receive monetary compensation for their diving activities. They are often unaware of the medical problems that may occur with diving. This pilot study examined professional diver knowledge of diving medical issues (DMI) through a questionnaire.

Methods

Twenty-six professional divers completed a 5-part diving experience questionnaire including ten statements on DMI. Four of the DMI statements reflected knowledge of general diving medical information (Group 1), three statements required specific diving medical knowledge (Group 2) and three statements dealt with unresolved diving medical controversies (Group 3). A single response from the following three choices: "agree" (A), "disagree" (D) and "not sure" (NS) was made for each statement.

Results

Professional divers showed a good knowledge of general medical issues in Group 1 responses: 88% correct, 5% incorrect and 7% NS. Controversial Group 3 statements had responses equally divided between the three choices: A=30 (39%), D:20 (26%) and NS:27 (35%). Responses to Group 2 statements regarding specific DMI were: 23% correct, 41% incorrect and 36% NS.

Conclusions

This pilot study reveals a lack of specific diving medical knowledge among professional divers. We feel this study should be expanded with a questionnaire given to each diver at the time of his or her interval diving physical examination. The diver's responses to the questionnaire should be discussed with him or her in order to make the diver as knowledgeable about medical aspects of professional diving activities as possible.

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Key Words

Occupational diving, underwater medicine.

CARBON MONOXIDE POISONING

Carbon monoxide poisoning in recreational diving: an uncommon but potentially fatal problem.

Caruso JL, Hobgood JA, Uguccioni DM and Dovenbarger JA. *Undersea Hyperbaric Med* 1998; 25 (Suppl): 52

Abstract

Background

Carbon monoxide (CO) is a colourless, tasteless, non-irritating gas that is produced by the incomplete combustion of hydrocarbons. It is the most common cause of death by poisoning in the United States. Extremely low levels of carbon monoxide arc normally present in the atmosphere, but great care is usually taken to avoid abnormally high amounts being introduced into scuba tanks. Increased lev-