Editorial

Endothelium: A Target for Therapeutic Intervention

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Endothelium, describes the wall of cell nuclei lining blood vessels that were first identified in 1845 by Todd and Bowman [1]. Three decades later Jones suggested the endothelium was, "… a continuous tube separated by a larger or smaller interval …." and "…consisting apparently of a very fine homogeneous membrane, bearing every here and there elongated fusiform nuclei" [2]. Using silver nitrate it was shown "…that this membrane is not truly homogeneous, but is made up of thin plates…. lessening friction and any tendency in the liquor sanguinis to coagulation, or to adhesion of the corpuscles to the wall" [2]. Subsequently, using transmission electron microscopy, these "thin plates" were shown to be physically distinct cells [3] that, in an adult human, may number up to 6 x 10^{13}, cover a surface area of ~1.7 m^2 and weigh ~1 kg [4]. Given these facts it is not surprising that the endothelium is now recognised as a "dynamic, heterogeneous, disseminated organ that possesses vital secretory, synthetic, metabolic and immunologic functions" [5].

1980 saw an expansion in endothelial research following the classic Nature paper of Furchgott and Zawadzki describing the obligatory role of endothelial cells in arterial smooth muscle relaxation caused by acetylcholine [6]. By 1987 Furchgott's potent endothelial derived relaxing factor (EDRF) was identified as the gaseous transmitter, nitric oxide (NO) [7, 8], with the potent endothelium-derived constrictor, endothelin (ET), discovered the following year [9]. Apart from their vasoactive properties NO and ET were shown to possess many other actions including cell proliferation, inflammation and neurotransmission. In addition to NO and ET other important endothelium-derived factors exist including prostaglandins [10, 11] and purines [12]. Given the number of such factors and their role(s) in homeostasis, the endothelium is recognized as a potential target for therapeutic intervention in many pathological conditions.

This 'Endothelium' Special Issue of *Current Vascular Pharmacology* includes reviews from experts in their field describing various aspects of endothelial research.

One such expert is Paul M. Vanhoutte [13] who, in his essay "Endothelial Lessons", refers to lessons learned over 30 years of endothelial research. Apart from providing an historical overview of the identification of various endothelium-derived factors (EDRF/NO, EDHF, EDCH and even IDNK: see essay for details), Vanhoutte exposes the many limitations of a variety of techniques commonly used in vascular research, limitations that are too often ignored. In particular, the final section of this essay, "Tissues don't lie!" discusses the importance of serendipity in research, especially when generating unexpected results. The lessons described in this essay are particularly relevant to young researchers embarking on their new careers.

In his review, Geoffrey Burnstock [14], outlines the involvement of purinergic signalling in the control of vascular tone and remodelling. Burnstock first proposed purinergic signalling in 1972 with receptors for ATP being characterised in the early 1990s. Vascular tone is regulated by sympathetic nerve vasoconstriction that is opposed by endothelium-mediated vasorelaxation where ATP is released from endothelial cells in response to altered blood flow and hypoxia. The actions of other locally released purine and pyrimidine nucleotides and adenosine are also involved in the regulation of vascular tone. Apart from ATP being released from endothelial cells, endothelial A2A and/or A2B receptors and 'P2X/P2Y' receptors mediate vasodilatation. Other endothelial purine release mechanisms, receptors and ecto-nucleotidases are discussed that represent potential targets for drug development.

Mustafa Zakkar and colleagues [15] provide a detailed overview of the regulation of vascular endothelium inflammatory signalling by shear stress, an important stimulus mentioned by other contributors to this special issue. The authors describe how shear stress plays a role in regulating endothelial cell inflammatory responses and the pathogenesis of atherosclerosis with particular reference to the regulation of MAPK and NF-κB. Blood vessels are influenced by many forces such as hydrostatic pressure, traction, and distension. However, shear stress is considered the most important mechanical force that can influence blood vessels *via* various aspects of endothelial cell physiology including the regulation of vasoactive substances, growth factors and adhesion molecules. In particular, shear stress plays a pivotal role in atherogenesis that may be related to site-specific suppression of inflammation.

The review by Napachanok Mongkoldhumrongkula, Magdi Yacoub and Adrian Chester [16] discusses the endothelial cells covering the heart valves. Each surface of the valve is exposed to vastly different patterns of blood flow and levels of shear stress and, in this article, the authors describe the role of endothelial cells on either surface of the heart valves and how they contribute to the valve's function and durability. Endothelial cells that lie on the aortic side encounter low oscillatory shear stress whereas those on the ventricular side are subjected to high laminar shear stress. These differences may affect heart valve endothelial cell morphology and biology. Since endothelial cells from the opposite sides of the valve are subjected to different types of flow they exhibit divergent gene expression on factors that protect the valve against pro-calcification mediators and those which influence the mechanical properties of the valve. Compelling evidence exists that valve endothelial cells not only

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differ from other endothelial cells in the cardiovascular system but that they possess specific properties depending on their aortic or ventricular valve location. Future studies are required into the development of heart valve endothelial cells, the mechanisms involved when these cells become dysfunctional and how the progression of valve disease is mediated.

Christian Jung and John Pernow [17] review the role of endothelial dysfunction in the pathogenesis of vascular disease in diabetes mellitus and impairment of NO bioavailability. Upregulation of arginase is associated with reduced NO bioavailability due to competition for the substrate, L-arginine, between arginase and endothelial NO synthase. Arginase upregulation is also associated with increased oxidative stress causing further impairment of NO bioavailability and therefore suggested to be a key factor involved in endothelial dysfunction in diabetes.

Chris Blick, Robert Ritchie and Mark Sullivan [18] discuss the role of endothelial cells dysfunction in erectile dysfunction (ED), a condition affecting approximately half of men during middle age. The pathophysiology of ED and cardiovascular disease is closely linked with endothelial dysfunction occurring at early stages of both conditions. ED disrupts the regulation of smooth muscle contraction and penile vascular tone with reduced NO bioavailability occurring in response to endothelial damage. A number of endothelium-derived, NO independent, factors also contribute to ED in the presence of endothelial damage. Targeting these pathways and attempts to reverse endothelial damage remain the standard medical therapy for ED.

Lei Shen *et al*. [19] provide novel data regarding the potential role of endothelium-derived adiponectin, a factor generally associated with adipose tissue. As such, adiponectin is an adipokine that is reduced in obesity and increases on weight loss. It is also anti-inflammatory, promotes insulin sensitivity and affords cardiometabolic protection. Although considered a true adipokine, in that it is exclusively generated by the adipocytes, there is recent evidence that adiponectin is secreted by a range of other organs. This review summarizes the non-adipose sources of adiponectin especially that derived from the endothelium. The vasoprotective role(s) of adiponectin suggest that it may be a novel target for clinical intervention in cardiovascular disease.

In his contribution, Anil Gulati [20] provides an overview describing how endothelial functions are altered following hypovolaemic shock. As a major site of metabolic activity and major reservoir of multipotent stem cells the endothelium plays a vital role in vascular physiological and reparative processes. In addition, the endothelium is involved in various pathological conditions including dengue shock syndrome and Ebola haemorrhagic fever. Endothelial functions are altered following hypovolaemic shock due to ischaemia of endothelial cells and by reperfusion due to resuscitation with fluids *via* endothelial cell activation and the release of vasoactive substances, mediators of inflammation and thrombosis. A greater understanding of these functions and mechanisms will provide the ability to design therapeutic agents for the acute management of hypovolaemic shock due to blood loss following trauma such as major injuries on the battlefield as well as the treatment of patients with dengue shock syndrome and Ebola haemorrhagic fever.

Most reviews in this endothelium special issue describe how a variety of drugs that have been developed and tested in experimental models have migrated from 'bench to bedside'. In particular, those targeting the ET and NO systems have shown the most promise with both ET receptor antagonists and phosphodiesterase type 5 inhibitors presently used in the treatment of pulmonary arterial hypertension [21]. Although many experimental studies indicate that a number of conditions may be treated with such drugs they have not yet reached 'the bedside'.

There are a variety of areas where promising experimental endothelial data have not been pursued, the endothelin field in particular presents many illustrations. Our own experience has been in studies into the therapeutic potential of ET receptor antagonists in reducing neointimal thickening and luminal narrowing in porcine models of vascular damage caused by balloon angioplasty and an experimental model of bypass surgery. For example, neointimal thickening that occurs following balloon angioplasty and coronary artery bypass grafting in patients with coronary artery disease has serious consequences. Angioplasty-induced neointima formation [22] as well as that in a pig vein graft model [23] is dramatically reduced in animals receiving an ETA receptor antagonist. If such results were obtained in coronary artery disease patients, the use of ETA receptor antagonists (possibly in combination with other drugs such as aspirin) may prove effective in reducing restenosis following angioplasty and coronary artery bypass surgery.

A role for ET-1 in Alzheimer's disease (AD) has been suggested, based on experimental evidence in rats where both ETA and ETB receptor antagonists affect beta amyloid (Aβ)-induced oxidative stress and cognitive impairment [24, 25]. Further evidence for an involvement of ET-1 in Alzheimer's disease has been described where Endothelin Converting Enzyme-2 and ET-1 is elevated in postmortem temporal cortex from AD patients [26, 27]. Despite evidence for a role of ET in AD and the potential beneficial effect of ET receptor antagonists, none of these drugs have yet undergone clinical trial as far as I am aware.

In this Endothelium Special Issue, Blick *et al*. [18] list ET-1 among the candidates involved in erectile dysfunction (ED). ET-1 antagonists may prove beneficial in the treatment of ED, particularly when used in combination with agents which are established for the treatment of ED. This suggestion has been made in the review by Khan *et al*. [28] yet, to my knowledge, ET antagonists have not yet been used in patients with this condition. Interestingly, there is also evidence that ET-1 may play a role in female sexual dysfunction (FSD), a condition where vaginal and clitoral blood flow may be an important pathological example where ET-1 levels are elevated. This experimental evidence is based on organ bath studies where ET-1-induced contractions obtained on segments of female rat pudendal artery were reduced in the presence of an ETA receptor antagonist or Rho-kinase inhibitor [29]. As in male ED, diabetes is a risk factor for FSD. *In vitro* studies such as those described previously were performed on Goto-Kakizaki (GK) rats, a non-obese model of type 2 diabetes with elevated ET-1 activity. Here, internal pudendal

arteries, but not mesenteric arteries, from GK rats exhibited greater contractile sensitivity to ET-1 compared with control arteries (Wistar rats) with both being reduced by an ETA receptor antagonist. Again, Rho-kinase inhibition reduced ET-1-mediated constriction of GK internal pudendal arteries and RhoA protein expression was elevated. Unlike 'male ED', FSD receives little attention and the authors' findings provide important evidence in support of a vasculogenic component in this condition and raised sensitivity of the internal pudendal artery in diabetes to certain factors, including ET-1 [30].

ET-1 is recognised as a factor involved in diabetic complications of the vasculature [31]. A study comparing leg muscle biopsies from patients with critical limb ischaemia (CLI) versus controls revealed an increased microvessel density of approximately two and a half fold (angiogenesis) in patient samples and an increase in ETB receptor density as well as ETB receptor mRNA expression [32]. It was concluded that, in CLI, ET-1 may mediate angiogenesis and NO release *via* ETB receptors in microvessels of ischaemic muscle. In a subsequent study ET-1 was shown to be associated with regions of atherosclerotic plaque in popliteal arteries from patients with CLI as well as with endothelial cells of the main vessel lumen and adventitial microvessels. ETA and ETB receptors were also located at these sites. There was a statistically significant increase in plasma ET-1 in CLI patients when compared with controls. Taken together these results were suggested to identify sources of ET-1 in atherosclerotic popliteal arteries that contribute to the increased circulating levels of this peptide in patients with CLI and that there may be therapeutic potential of selective receptor targeting in patients with this condition [33]. There is data to support ETA receptor blockade having a beneficial effect in patients with type 2 diabetes and CLI. Here, the effect of ETA receptor blockade on peripheral micro- and macrocirculation in patients with type 2 diabetes and CLI was studied measuring systolic toe blood pressure and transcutaneous oxygen tension at the first intermetatarsal space of the foot after 30 min of acclimatization. A percutaneous catheter was inserted into the femoral artery with toe blood pressure and transcutaneous oxygen tension being re-evaluated after a 60-min infusion of ETA receptor antagonist [34]. The results indicated that ETA receptor blockade has a beneficial effect on local tissue perfusion in patients with type 2 diabetes and CLI, an effect due to blocking precapillary ET-1 mediated constriction *via* ETA receptors as well as improved endothelium-dependent, ETB receptor/NO-mediated vasodilation. Again, an indication that targeting the ET-1 system might be of importance in the treatment of complications related to diabetic angiopathy.

Not long after its isolation ET-1 was implicated in various forms of cancer. For example, ET-1 immunostaining and ET-1 mRNA have been identified in sections of squamous cell carcinomas and adenocarcinomas of surgically resected lung tumours [35]. Using quantitative receptor autoradiography high affinity radiolabelled ET-1 binding was identified that was localized in the blood vessels of pulmonary tumours where it was suggested that ET-1 may play a role in the angiogenesis of tumour growth [36]. The tumorigenic action of ET-1 is primarily believed to be through ETA receptor activation and, in preclinical models, endothelin antagonism inhibits tumour cell proliferation, invasiveness and new vessel formation with orally active endothelin antagonists demonstrating benefit in prostate-specific antigen progression, markers of bone turnover and pain in men with prostate cancer. However, at the time of this review no significant improvement in survival or time to cancer progression could be demonstrated [37]. In their article, Bhalla and colleagues [38] provide an overview of the involvement of ET-1 in the development of several tumours with reference to studies into the role of ETA and ETB receptors and the potential therapeutic potential of receptor antagonists. A more recent review discusses the current interest in the role of selective ETA receptor antagonists citing studies implicating this subtype in the growth and progression of several tumours including prostate, ovarian, colorectal, bladder, breast and lung carcinomas. Here a useful discussion is provided regarding pathways involved in cell proliferation, migration, invasion, epithelial-mesenchymal transition, osteogenesis and angiogenesis as well as emerging preclinical and clinical data, in particular in patients with prostate cancer, where there are encouraging results for ETA receptor antagonists [39].

Many ET receptor antagonists have been produced yet, at present, the bedside applications are limited. Given the number available and the vast amount of published experimental data, this is disappointing and, although the focus of this editorial section has been on endothelin, the same might be said of many other endothelium-derived factors.

Hopefully, advances in drug delivery [40] and tissue targeting [41, 42] may improve the therapeutic potential of drugs acting on the endothelium advancing them from the bench to the bedside.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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