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Editorial

Perspectives in pharmacology of endothelium: From bench to bedside

The vascular endothelium, hitherto regarded as a passive blood-compatible, semi-permeable membrane resembling nucleated cellophane [1] has over the last decades been recognised as a dynamically regulated organ that is critical in maintaining vascular homeostasis. In contrast to other vital organs the endothelial organ is composed of only one type of cells. Yet, it is composed of a tremendously heterogeneous — structurally and functionally — population of cells (1 to 6×10^{13}) that is disseminated throughout the body to cover huge inner surface (4000 m^2) of the entire cardiovascular system. This single layer of endothelial cells ($\leq$1 μm in thickness) is involved in an impressive array of functions, including the regulation of permeability and transfer of substances and macromolecules, the regulation of vascular tone, vascular structure and new blood vessel formation. Endothelium is also critical in the regulation of the body response to pro-inflammatory and pro-thrombotic signals because it regulates adhesion and aggregation of platelets, coagulation, fibrinolysis, trafficking of white blood cells, innate and acquired immune response [2].

Most importantly, impairment of endothelial function is involved in most if not all human diseases either as a primary determinant of pathophysiology of disease or as a victim of collateral damage that can amplify the pathophysiology of a disease. Abundant literature provides compelling evidence for the important role of endothelium in numerous human diseases including atherothrombosis, diabetes, heart failure, sepsis, pulmonary hypertension, neurodegenerative diseases, liver steatosis, cancer metastasis and many others [3–11].

Today, a couple of decades after seminal discoveries of the first endothelial mediators such as prostacyclin (1976) and EDRF (1980) later identified as NO (1986) [12–14] that were followed by subsequent discoveries of hundreds of endothelial mediators, we are fully aware of the embarrassing richness of endothelium-dependent mechanisms that may contribute to initiation and progression of various diseases. However, translating this gigantic mass of knowledge accumulated over years into the endothelium-guided diagnostics and endothelium-targeted therapy of diseases is not that simple. Firstly, there are a couple of limitations to our understanding of endothelial physiology and pathophysiology including the incredible complexity and heterogeneity of endothelium and the paucity of relevant experimental methods to study microvascular endothelium in microenvironments recapitulating natural microvascular endothelial setting *in vivo* (presence of neighboring cells, physioxia and flow conditions). Furthermore, although it is evident the assessment of endothelial function has diagnosis, prognostic and therapeutic significance [4,5,8,9], the methods available to diagnose endothelial function in clinical setting are not suited for everyday clinical practice [15]. Finally, even though there are endless novel opportunities to develop novel endothelium-targeted therapeutics for a variety of diseases, their clinical proof-of concept studies would require a validated method for assessing endothelial function that could be used as a reliable surrogate end-point of a disease progression and efficacy of endothelium-targeted therapy.

Altogether, despite more than 300,000 published articles related to endothelium there is still a huge bench-to-bedside gap in endothelial biomedicine, the discipline that grateful to William C Aird has got its first comprehensive textbook recently [16].

The present special issue of *Pharmacological Reports* was aimed to overview some of the advances and challenges in endothelial biomedicine at experimental and clinical levels. The contributions were divided into four sections addressing novel pharmacotherapeutic mechanisms of endothelium [17–24], novel methodologies to study endothelial function [25–30], strategies for comprehensive assessment of endothelial function in humans [31–35], and finally the endothelial toxicity of drugs or endogenous metabolites [36,37].

Novel pharmacotherapeutic mechanisms of endothelium

For decades, nitrites (NO_2^-) and nitrates (NO_3^-) were thought to be stable end products of nitric oxide (NO) produced by NO synthases (NOS). Recently, however, it has been proposed that the NO_3^-–NO_2^-–NO pathway acts as a backup system for NO generation in situations in which the endothelial NO pathway is compromised. Quite surprisingly, xanthine oxidoreductase (XOR) catalyzing the final steps in purine catabolism and a known source of reactive species, may also act as a nitrite reductase. Kelley [17], review the microenvironmental conditions requisite for *in vivo* XOR-catalyzed oxidants and NO formation providing a novel insight into biochemistry and pharmacology of vascular XOR.

In turn, ecto-5′-nucleotidase (CD73), an extracellular enzyme of nucleotide catabolism that is highly abundant in endothelium converts extracellular AMP to adenosine, which plays a role of a negative regulator of pro-thrombotic and pro-inflammatory activation of endothelium. As discussed by Zukowska et al. [18] downregulation of CD73-derived adenosine contributes to dysfunctional endothelium phenotype, while pharmacological activation of this pathway represents a novel strategy for improving endothelial function.

Adenosine availability in the endothelial microenvironment is regulated not only by CD73 but also by intracellular AMP

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deaminase (AMPD) that converts AMP to IMP. Inhibition of AMPD represents another interesting approach to boosting endothelial adenosine, which due to concomitant elevation of intracellular AMP also leads also to the activation of AMP-regulated protein kinase (AMPK). Zabielska et al. [19] review the endothelium-oriented therapeutic potential of AMPD inhibition resulting in the activation of two concomitant pathways: adenosine- and AMPK-dependent, both of which improve endothelial function.

NO is a major hepatoprotective mediator produced by liver sinusoidal endothelial cells (LSEC), a structurally and functionally unique type of fenestrated endothelium. There is accumulating evidence that NO maintains LSEC fenestration and liver integrity and regulates lipid and glucose homeostasis in the liver. Maslak et al. [20] discuss the evidence that impairment of LSEC-derived NO-dependent regulation contributes to the development of Nonalcoholic Fatty Liver Disease (NAFLD). The authors claim that liver selective NO-based drug therapy exemplified by a notable example of anti-steatotic efficacy of V-PYRRO/NO represents a novel possible therapeutic approach to treat NAFLD.

Numerous mechanisms that regulate fibrinolysis including tissue type plasminogen activator (t-PA), urokinase type plasminogen activator (u-PA), and their inhibitor plasminogen activator inhibitor (PAI-1), plasmin and its inhibitor α-2-antiplasmin and thrombin activable fibrinolysis inhibitor (TFPI) that inhibits the binding of t-PA to fibrin. Kramkowski et al. [21] investigate how major endothelial mediators such as PGI_2, NO, and CO regulate fibrinolysis and provide convincing evidence that many cardiovascular drugs, including RAS inhibitors and statins enhance fibrinolysis *via* endothelium-dependent mechanisms that may contribute to the therapeutic benefits of these major cardiovascular drugs.

Recently, it has become apparent that alterations in endothelial metabolism are closely linked to changes in endothelial phenotype. Since anaerobic glycolysis but not oxidative phosphorylation represents a major source of ATP production in endothelium, mitochondria may rather play an important signaling role in these cells. Szewczyk et al. [22] review the mitochondrial mechanisms of endothelial dysfunction that could be targeted in endothelial pharmacology focusing on the role of mitochondrial ROS, uncoupling proteins (UCP) and mitochondrial potassium channels, including BK_{Ca} in the regulation of endothelial phenotype.

The last two articles in this section examine the potential of endothelial-targeted pharmacology in cancer metastasis and exercise pathophysiology. Blazejczyk et al. [23] explore endothelial mechanisms that accompany the progression of cancer metastasis from early infiltration of tumor cells through the endothelial layer until the development of vascularized metastatic foci. The authors look at various endothelium-oriented therapeutic approaches that may afford anti-metastatic activity, including PGI_2-releasing molecules that have been proven to inhibit cancer metastasis. Treatment of cancer metastasis is still "unmet medical need" and current efforts seem to be concentrated on the development of biological therapies including anti-angiogenic agents. Endothelial-targeted therapy aimed at modulating anti-adhesive properties of endothelium may thus represent a novel approach to combatting metastasis. In turn, the contribution by Zoladz et al. [24] describes the current state of knowledge on mechanisms of endothelium-dependent regulation of coronary and skeletal muscle blood flow and exercise capacity, focusing on NO, PGI_2 and erythrocyte-released ATP. Clearly, maladaptation of these mechanisms may not only impair exercise capacity but may also increase the cardiovascular risk of exercise. These facts open a novel and important field in endothelial pharmacology aimed at modulating maladaptive endothelial response during exercise to maximize the health benefits of exercise and to limit its cardiovascular hazards.

Novel methodologies to study endothelial function

Undoubtedly, the application of novel methodologies in endothelial biomedicine may bring novel understanding of endothelial function. Atomic force spectroscopy (AFM) and Raman spectroscopy are notable examples of experimental techniques that give insight into endothelial biology that is additive to those given by other methods. Szymonski et al. [25] discuss an AFM-based approach to studying endothelial cells. This is a relatively new technique that allows not only to characterize endothelial surface topography, but also provides high resolution, quantitative determination of nanomechanical properties of a single endothelial cell and its subcellular compartments (e.g., cortical layer). Furthermore, AFM-based tip force spectroscopy is a unique method for studying the functional integrity of endothelial glycocalyx. Interestingly, the nanomechanical properties of endothelium are tightly linked with its biochemical phenotype because dysfunctional endothelium characterized by impaired NO production is "stiff", while healthy endothelium with preserved NO formation is "soft".

In turn, Raman spectroscopy represents the unique method that can characterize non-invasively, in a non-destructive way, and a quantitative manner biochemical contents of endothelium with high subcellular resolution and 3D confocal imaging. Two contributions, by Baranska et al. [26] and Marzec et al. [27] provide complementary perspectives summarizing proof-of concept studies on the application of Raman spectroscopy to detect endothelial dysfunction and vascular pathology in *ex vivo* vessels isolated from diabetic, hypertensive and atherosclerotic mice. The evidence supports the unique potential of Raman spectroscopy to reveal disease-specific alterations in biochemical signature of dysfunctional endothelium. The authors highlight future directions and a vast array of possible applications of Raman spectroscopy-based method to studying biochemical changes in a single endothelial cell examined under physiological conditions.

Nanotechnology represents an important new avenue in endothelial biomedicine that, thanks to the development of multifunctional nanoparticles, may offer the detection of endothelial pro-inflammatory and pro-thrombotic phenotype and provide endothelium-targeted drug delivery in a personalized manner. Advantages and limitations of endothelial theranostics are reviewed by Zapotoczny et al. [28].

It is widely known that excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) contributes to endothelial dysfunction, and their specific detection and real-time quantification in a living endothelial cell remains a challenge. Debowska et al. [29] present a comprehensive review of mechanistic and quantitative aspects of ROS and RNS detection using classical and novel fluorogenic probes. The authors present a concept of the "global profiling approach" for simultaneous real-time monitoring of various ROS and RNS species that should provide an excellent platform for better understanding of the role of ROS/RNS in endothelial function and dysfunction.

Endothelial dysfunction is featured by increased permeability, impaired NO-dependent vasodilation and increased expression of various pro-adhesive molecules on endothelial surface. Bar et al. [30] summarize the MRI-based method for assessing endothelial function in mice based on biochemical phenotype and molecular imaging but primarily based on the assessment of endothelium-dependent vasodilation and changes in endothelial permeability. The methodology described in this review should prove extremely useful in *in vivo* proof-of concept studies of the mechanisms and efficacy of endothelium-targeted therapeutics in mice models of numerous diseases associated with endothelial dysfunction.