Measurement of microcirculation in clinical research

Birkhoff, W.A.J.

INTRODUCTION

Measurement of microcirculation in clinical research

Cardiovascular disease is still one of the most important causes of death, with 17.7 million deaths in 2015, although this has dropped substantially in the last decades.[1]This was mainly caused by emerging treatment options initially driven by the Framingham studies which identified hypertension and hyperlipidemia as risk factors for cardiovascular disease. This resulted in the development of pharmacological treatments which are still widely used. Currently, a treatment option is available for most macrovascular diseases. However, interventions targeting the microcirculation are few and far between. Evaluation of treatments affecting the microcirculation is difficult because of absence of standardized accepted techniques Moreover, it has only become apparent recently that large macrovascular diseases are preluded by microvascular abnormalities and microvascular measurements were strong predictors of long-term cardiovascular disease and survival.[2–4] Therefore, early intervention targeting the microvascular abnormalities may support preventing later onset of cardiovascular problems thereby reducing the impact on global heath.

Microcirculation anatomy

The systemic circulation delivers blood to all organs and body tissues, amongst others exchanging oxygen and carbon dioxide during its course. Exchange takes place mostly in the capillaries, which are about 5 to 10 µm in diameter, are lined with endothelial cells, and are one layer thick. Despite their small size, the surface area of the capillaries is the largest of all vessels in the circulation. Together with the arterioles, venules, lymphatic capillaries and collecting ducts, these vessels form the microcirculation.

Physiology

Blood flow in the (micro)circulation is mainly regulated by the arterioles. The arterioles can adjust their diameter and vascular tone by contracting and relaxing the vascular smooth muscle wall. The endothelial tissue in the vessels responds to various stimuli, including neuropeptides, neurotransmitters, several hormones, and distension of the vessels due to increased blood pressure.[5] These stimuli have an effect on the complex interplay of hormones secreted by the endothelial tissue, the main hormones being nitric oxide (NO) and endothelin-1 (ET-1). NO is a gaseous hormone, which is generated by endothelial nitric oxide synthase (eNOS), which is in its turn activated by acetycholine, bradykinin or serotonin.

NO is secreted from the endothelial layer and then travels to the smooth muscle cells, where the NO triggers membrane-bound - and soluble guanylate cyclases, which in its turn synthesizes cyclic guanylate monophosphate (cGMP), after which cGMP-kinase is activated. By stimulation of K+-channels and thereafter inhibition of the Ca++-channels – this enzyme lowers intracellular Ca++ [6] and subsequently relaxes the smooth muscle cells. However, nitric oxide has various other functions including platelet adherence, leucocyte chemotaxis and smooth muscle cell proliferation.[7,8] Endothelin-1 has the exact opposite effect from NO, although its effects have not been as well described. Predominantly through up- and downregulation of these hormones, the endothelial tissue can regulate wall stress.

Pathophysiology – Endothelial dysfunction

Endothelial dysfunction is the harmful alteration of endothelial physiology, thereby affecting blood flow and vascular homeostasis. The main mechanism by which these alterations have an effect is by reducing the bioavailability of NO. For example, hypercholesterolemia induces a mild inflammation of the vascular wall and generates O_2 radicals. These O_2 radicals react with NO to form peroxynitrie ($ONOO^-$), reducing the vascular relaxation effects of NO. Peroxynitrite in itself modulates the NO generation by inactivating eNOS, through modification of the Zn^{2+}sulfur motifs in the eNOS protein,[9] even further reducing the bioavailability of NO.[10] Endothelial dysfunction is of pathophysiologic importance in atherosclerosis and has been shown to be a strong predictor of atherosclerotic cardiovascular disease. In several studies, endothelial dysfunction, measured by flow mediated dilation, was observed before any other indication of atherosclerosis.[11] This has led to various research initiatives on improving endothelial function and thereby alleviating atherosclerosis. Endothelial dysfunction is associated with cardiovascular risk factors for atherosclerosis, such as diabetes, dyslipidemia, hypertension, smoking and aging. In the clinic, early detection and correction of endothelial dysfunction may therefore play an important role in preventing these (irreversible) conditions.

Interventions

Clinical studies have observed that endothelial function can be improved by treatment with angiotensin converting enzyme (ACE) inhibitors,[12] antioxidant agents,[13,14] beta blockers,[15] calcium channel blockers,[16] phosphodiesterase (PDE) 5 inhibitors[17] and statins.[18]

These interventions cause indirect antioxidant effects (e.g. prevention of eNOS uncoupling) and through their anti-inflammatory improve the functioning of the endothelium. This exact mechanism is extensively described.[12] Briefly, the antioxidant effects consists of three components:

1 ACE inhibitors and and angiotensin-II receptor antagonist increase the NO bioavailability by decreased bradykinin breakdown
2 hydrogen peroxide, cellular superoxide, and peroxynitrite levels are decreased by avoiding the activation of the Nox2 enzyme
3 ACE inhibitors inhibit the development of diacylglycerol, a induction of NADPH-oxidase activity. The anti-inflammatory effect is caused by interruption of monocyte adhesion to the endothelium.[12]

Most studies regarding these mechanisms have been performed in knockout mice, or by measuring FMD in patients, although more recently microvascular measurements have been implemented.[19]

Although these treatments are widely available and have been shown to reduce cardiovascular complications, there is a need for evaluation of the effect of these and new pharmacological therapies on microvascular endothelial dysfunction. There is increasing evidence that microvascular dysfunction is not only a consequence of atherosclerosis but also an independent risk factor for vascular events

Interest into evaluation

The microvasculature is considered sensitive to various stimuli, from vascular, immunological, metabolic and neurologic origins, making the microvasculature a final common pathway for many (pharmacological) interventions. Combined with its excellent accessibility and relatively non-invasive measuring tools, since microvascular evaluation can be performed on lower forearm skin or in the eyes, further evaluation to identify the possible applications for microvasculature models is warranted. In particular interest for this thesis is the application of microvascular models to evaluate novel compounds in healthy volunteers.

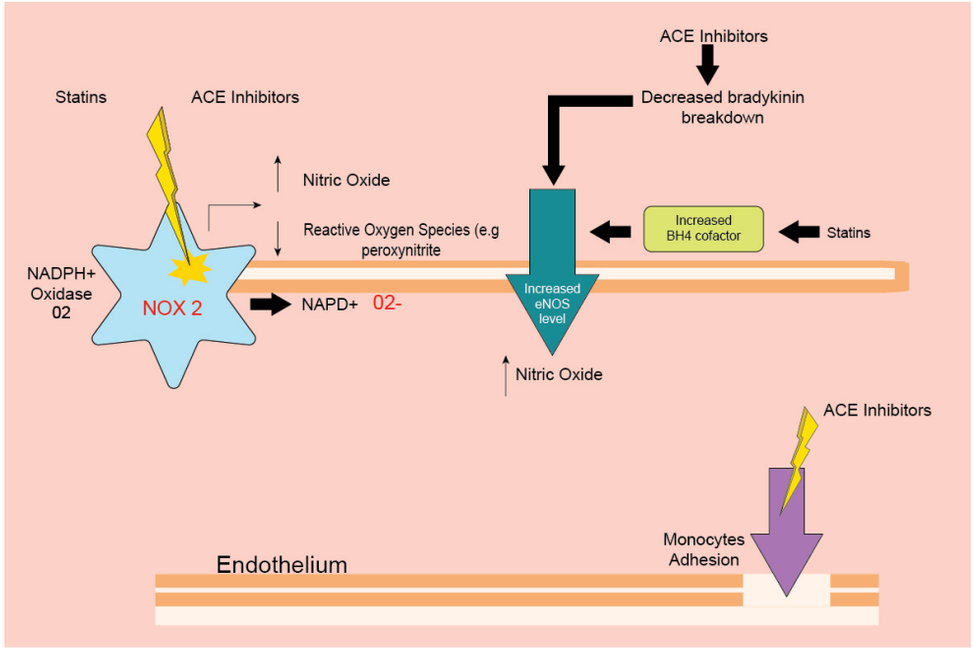
Validation

The overall aim of this thesis is to perform the first steps into validating multiple tools to identify pharmacological or disease effects on the microvasculature. To do this, the CHDR model for validation is applied. The CHDR algorithm is an algorithm which novel devices must complete before they can be implemented in routine clinical care or clinical studies. The algorithm consists of the following steps:

1 Analytical validation (EG intra/inter subject/measurement repeatability)
2 Clinical validation: demonstrating an association between the device output and disease activity, disease severity, or another area of interest; which is divided in the following substeps:
   - Can devices discriminate between clinical and healthy populations?
   - Can devices detect various event types, like known (negative) rigorous interventions (i.e. admission vs recovery)?
   - Can you correlate validated or traditional outcomes with the device parameters?
   - Performance qualification: Does a (known) effective health care intervention provide a comparable output with the device's parameters?
   - Practical usability in clinical practice.

After the device completes all steps successfully, the device is ready to be used in clinical care or studies, but only for the purpose that was identified in step 1.

FIGURE 1 Schematic representation of the endothelium and pharmacological targets. The image highlights potential sites for drug intervention.



This thesis

The present thesis investigates multiple novel devices that can perform measurements of microvascular function. We have investigated several new devices that have been developed or are that are currently under development and we investigated the applicability of several novel microcirculation measurement devices for their utility in the clinic or in a clinical research setting. These techniques are more extensively described in the next chapter.

For early phase drug development, studies are performed in healthy volunteers. Therefore, non-invasive access to evaluate the microvascular function is key, and therefore we have chosen to validate devices that are able to measure the microcirculation in either the skin or in the eye. As these organs are relatively accessible and the measurements non-invasive, but nevertheless can potentially be very informative about general microcirculation without harming the study subject. Most measurements in these organs can be performed with relative comfort for the patient and as such are suitable for frequent application in the clinic or in clinical studies.

Chapter 3 focuses on validating the ability of the devices to distinguish between sickle cell patients and healthy volunteers, given that they were relatively new at the time. Sickle cell disease is a genetic disorder in which a single point mutation leads to abnormal synthesis of hB (HBS). This HBS can lead to abnormal folding of hB chains and subsequently formation of sickle cells. Besides painful and invalidating sickle cell crises, sickle cell patients have severe vascular and rheological abnormalities, leading also to an impaired endothelial function. [20]

In **chapter 4,** cutaneous microvascular measurements were compared to cerebral flow measurements in sickle cell patients, with the aim of validating the device to measure cerebral flow. Next to applications in early phase drug development, for example for drugs that aim to increase cerebral blood flow, the device has potential to be an alternative for expensive and time-consuming MRI measurements.

In **chapter 5**, new techniques and analyzing methods were evaluated for retinal microvascular measurement, including fractal analysis which can be used to measure microvascular function in retinal vascular beds and multispectral imaging in the retina. Also, in **chapter 8**, multispectral imaging was evaluated in the skin in heathy volunteers, aiming to validate the technique for further use. In **chapter 6**, Micro Dynamic light scattering was evaluated for the applicability in clinical and research use, by evaluating the robustness of the MDLS by benchmarking it against other microvascular imaging techniques.

Finally, in **chapter 7**, we evaluated if the microvascular techniques can be used to evaluate the effects of a pharmacological intervention affecting the blood's rheological characteristics before and after exercise.

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