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Endothelial Dysfunction as an Early Sign of Atherosclerosis

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Key Words:
Endothelium · Risk factors · Atherosclerosis · Nitric oxide · Flow-dependent vasodilation · Prognosis · Endothelial progenitor cells

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Abstract

The endothelium, the monolayer covering the inner surface of blood vessels, plays a pivotal role in the regulation of vascular tone and structure, as well as vascular inflammation and thrombosis, i.e., of key events of the atherosclerotic disease process and its clinical complications, such as myocardial infarction and stroke. In particular a reduced endothelial availability of nitric oxide (NO), in part due to increased vascular oxidant stress, has been shown to promote a pro-inflammatory and prothrombotic phenotype of the endothelium. More recently, it has been observed that cardiovascular risk factors reduce the number and impair the function of circulating bone marrow-derived endothelial progenitor cells (EPCs), thereby impairing the endogenous endothelial repair capacity.

Importantly, endothelial dysfunction has been identified as a common link of all cardiovascular risk factors. Numerous clinical studies have further demonstrated a close association of the degree of endothelial dysfunction with the risk of future cardiovascular events. Whether endothelial dysfunction can improve cardiovascular risk prediction on top of a careful evaluation of classic cardiovascular risk factors is currently prospectively analyzed in several studies, i.e., in the PREVENT-it study. Furthermore, novel easier to use methods to assess endothelial function are currently explored, i.e., the Endo-PAT system, for their potential in improving cardiovascular risk prediction.

At present, assessment of endothelial function and EPCs are highly valuable research tools to improve our understanding of mechanisms of vascular disease and to determine the impact of novel therapeutic approaches on vascular function. Before endothelial function measurements can, however, be recommended in clinical practice for cardiovascular risk assessment, the results of ongoing prospective studies assessing the additive value of these measurements for cardiovascular risk prediction should be awaited.

Bedeutung der Endotheldysfunktion als Frühzeichen der Atherosklerose

Schlüsselwörter:
Endothel · Endothelfunktion · Kardiovaskuläre Risikofaktoren · Atherosklerose · Stickstoffmonoxid · Endotheliale Progenitorzellen

Zusammenfassung

Das Endothel, die einzellige Zellschicht, welche das Lumen der Blutgefäße auskleidet, spielt eine entscheidende Rolle bei der Regulation des Gefäßtonus und der Gefäßstruktur. Des Weiteren besitzt das „gesunde" Endothel wichtige antiinflammatorische und antithrombotische Eigenschaften. Kardiovaskuläre Risikofaktoren führen insbesondere über eine verminderte Verfügbarkeit des endothelial gebildeten Vasodilatators Stickstoffmonoxid (NO), zumindest z.T. als Folge einer gesteigerten vaskulären Produktion von Sauerstoffradikalen, zu einem proinflammatorischen und prothrombotischen Phänotyp des Endothels. Dies dürfte eine wichtige Bedeutung für die Entstehung der Atherosklerose und deren klinische Komplikationen, wie Herzinfarkt und Schlaganfall, haben. Die Induktion einer Endotheldysfunktion ist allen kardiovaskulären Risikofaktoren gemeinsam. Außerdem konnte in den letzten Jahren beobachtet werden, dass die kardiovaskulären Risikofaktoren zu einer verminderten Zahl und Funktion von zirkulierenden endothelialen Progenitorzellen führen, was die endotheliale Reparaturkapazität beeinträchtigen kann.

Mehrere klinische Studien konnten zeigen, dass die Ausprägung der endothelialen Dysfunktion, in der Regel bestimmt als eingeschränkte endothelabhängige Vasodilatation, mit der Entwicklung kardiovaskulärer Ereignisse eng assoziiert ist. Inwiefern eine Bestimmung der Endothelfunktion die Prädiktion des kardiovaskulären Risikos zusätzlich zu einer genauen Evaluation der bekannten kardiovaskulären Risikofaktoren verbessern kann, wird gegenwärtig in mehreren Studien prospektiv untersucht. Dabei wird auch analysiert, inwiefern einfacher zu handhabende Messgeräte zur Bestimmung der Endothelfunktion (wie das Endo-PAT-System) für die kardiovaskuläre Risikoprädiktion geeignet sind.

Gegenwärtig liefert die Bestimmung der Endothelfunktion wertvolle Einblicke in die Pathophysiologie kardiovaskulärer Erkrankungen und ist geeignet, neue Therapiestrategien im Hinblick auf ihre vaskulären Effekte zu charakterisieren. Vor einem möglichen Einsatz der Endothelfunktionsmessung in der klinischen Praxis zur Beurteilung des kardiovaskulären Risikos sollten die laufenden prospektiven Studien abgewartet werden, die den additiven Stellen-

wert der Endothelfunktionsanalyse zusätzlich zur genauen Beurteilung der bekannten kardiovaskulären Risikofaktoren im Hinblick auf die kardiovaskuläre Risikoprädiktion untersuchen.

Introduction

The endothelium has been identified as a major regulator of vascular tone and remodeling as well as arterial inflammation and thrombosis, thus of key processes of the atherosclerotic disease process. Importantly, endothelial dysfunction represents a common link of all known cardiovascular risk factors, such as dyslipidemia, smoking, diabetes, hypertension, obesity, and mental stress. Endothelial dysfunction represents likely an important pathway, thereby cardiovascular risk factors promote the development and progression of vascular disease.

The crucial role of the endothelium for the regulation of vasomotion was recognized by the experiments of Robert Furchgott's group [7], who later was awarded the Nobel prize in 1998 together with Louis Ignarro and Ferid Murad, who described the L-arginine-nitric oxide-(NO-)cyclic guanosine monophosphate (cGMP) system as a major system mediating endothelium-dependent vasodilation. Endothelium-derived NO activates the guanylate cyclase of vascular smooth muscle cells to promote cGMP-dependent vasodilation (Figure 1). This system is constantly activated, in part by the shear stress caused by the blood flow over the endothelium, to keep the arteries in a dilated state. Interestingly, it has then been discovered that endothelium-derived NO not only promotes endothelium-dependent vasodilation, such as during physical exercise, but has important anti-inflammatory and antithrombotic effects, thereby actively suppressing leukocyte adhesion and arterial thrombosis. Experimental studies could then confirm that endothelium-derived NO prevents development of atherosclerotic lesions [11], and can therefore be considered an important endogenous antiathero-genic system.

Endothelial Functions

The Endothelium and Vascular Tone

Since the 1980s it has been well known that the endothelium produces endothelium-derived relaxing factors (EDRFs) [7], and in the subsequent years, NO has been identified as a major EDRF [9, 17]. NO is synthesized by the action of the endothelial nitric oxide synthase (eNOS) mediating vasodilation, i.e., in response to shear stress. Several other stimuli, such as bradykinin and acetylcholine stimulate endothelial NO synthesis, and have therefore been widely used to assess endothelium-dependent vasodilation (Figure 1).

Other important EDRFs include the endothelium-derived hyperpolarizing factors (EDHFs), such as produced by the EDHF synthase cytochrome P450 2C [5], that are particularly important mediators in the microcirculation. In addition, the cyclooxygenase/prostacyclin synthase system of the healthy endothelium releases the vasodilator prostacyclin.

The endothelium also produces vasoconstricting factors, such as endothelin, vasoconstricting prostanoids, and angiotensin II, that can be increased under pathophysiological conditions.

Notably, the healthy endothelium has an intact balance of the release of endothelium-derived vasodilating factors, i.e., NO, prostacyclin and EDHFs, and endothelium-derived vasoconstricting factors, i.e., endothelin, angiotensin II and thromboxane A_2.

On the other hand, under pathologic conditions, i.e., hypercholesterolemia, hypertension, coronary disease and smoking, the endothelium has a reduced availability of vasodilating factors, in particular NO,

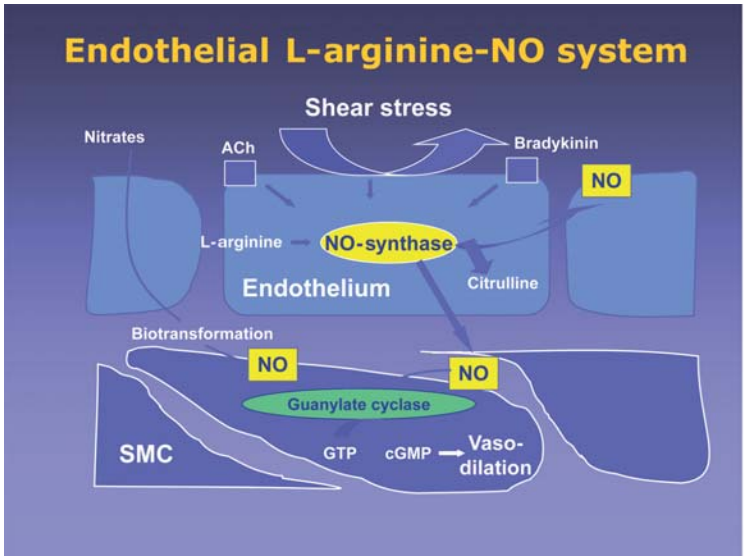

Figure 1. The L-arginine-nitric oxide (NO) system has been identified as an important endogenous vasodilatory system. The endothelial NO synthase produces NO from the amino acid L-arginine. Endothelial release of NO, such as stimulated by increased blood flow, activates guanylate cyclase in vascular smooth muscle cells leading to vasodilation.

Abbildung 1. Das L-Arginin-Stickstoffmonoxid-(NO)-System konnte als ein wichtiges endogenes endothelabhängiges Vasodilatationssystem charakterisiert werden. Die endotheliale NO-Synthase produziert Stickstoffmonoxid unter Verwendung von L-Arginin als Substrat. Das endothelial freigesetzte NO, z.B. durch den Blutfluss über dem Endothel stimuliert, aktiviert die Guanylatcyclase in den glatten Muskelzellen der Gefäßwand, was zur cGMP-abhängigen Vasodilatation führt.

and an augmented production of vasoconstricting factors, thereby impairing endothelium-dependent vasodilation, that is frequently assessed as "endothelial function".

The Endothelium and Vascular Inflammation

Atherosclerosis is considered a chronic vascular inflammatory disease today, and the activity of inflammation (i.e., C-reactive protein [CRP] measurements) has been related to cardiovascular risk [8]. Endothelial dysfunction promotes arterial inflammation and vice versa, chronic inflammation promotes endothelial dysfunction (Figure 2). This concept has been nicely illustrated by a recent study in patients with chronic periodontitis. After intense treatment of periodontitis associated with chronic inflammation, endothelial dysfunction was substantially improved, strongly suggesting that chronic inflammation promotes endothelial dysfunction [21]. Furthermore, in patients with coronary disease, endothelium-dependent vasodilation was closely related to serum levels of CRP [4].

Vice versa, on the molecular level, endothelium-derived NO production has been demonstrated to exert a major anti-inflammatory effect. Inhibition of eNOS stimulated the expression of leukocyte adhesion molecules and chemokines, such as monocyte chemoattractant protein-1, promoting vascular monocyte infiltration [3, 18], that is considered an important step in atherosclerotic lesion formation. Likewise, pharmacological inhibition of endothelium-derived NO production augmented endothelial adhesiveness of monocytes, further suggesting that a loss of endothelial NO availability promotes a pro-inflammatory phenotype of the endothelium.

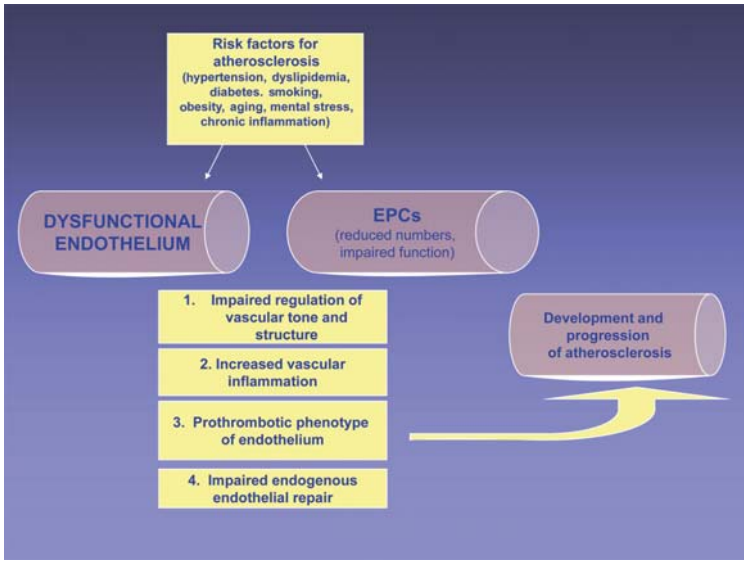

Figure 2. Cardiovascular risk factors and the inflammatory cascade act on the healthy endothelium to promote a pro-inflammatory and prothrombotic phenotype of the endothelium and impair the mobilization and function of endothelial progenitor cells (EPCs), mediated in part by a reduced endothelial cell and EPC nitric oxide (NO) availability due to increased vascular and systemic oxidant stress. The altered endothelium leads to an impaired endothelium-dependent vasodilation, and a pro-inflammatory and prothrombotic phenotype of the endothelium, promoting the development of atherosclerosis. Moreover, impaired EPC function alters the endogenous endothelial repair capacity.

Abbildung 2. Kardiovaskuläre Risikofaktoren und eine chronisch-entzündliche Aktivierung führen zu einem proinflammatorischen und prothrombotischen Phänotyp des Endothels und beeinträchtigen die Mobilisierung und Funktion endothelialer Progenitorzellen (EPCs), zumindest z.T. aufgrund einer Reduktion der endothelialen und EPC-NO-Bioverfügbarkeit als Folge einer gesteigerten Produktion von Sauerstoffradikalen. Die proinflammatorische und prothrombotische Aktivierung des Endothels führt nicht nur zu einer beeinträchtigten endothelabhängigen Vasodilatation, sondern begünstigt auch die Entwicklung und Progression der Atherosklerose. Die beeinträchtigte Funktion der EPCs vermindert sehr wahrscheinlich die endogene endotheliale Reparaturkapazität.

The Endothelium and Arterial Thrombosis

A healthy intact endothelial monolayer actively inhibits arterial thrombus formation by several ways. First, an intact endothelial layer is critical for protection of circulating blood from exposure to prothrombotic subendothelial material and matrix. Then, a healthy endothelium releases several important mediators to actively prevent platelet activation, adhesion, and aggregation. Notably, endothelium-derived NO limits platelet activation, adhesion and aggregation, and inhibits the expression of the prothrombotic protein plasminogen activator inhibitor-1 [14]. Moreover, platelets have been shown to express eNOS and to produce NO that likely limits recruitment of platelets to the platelet-rich thrombus. An impaired platelet-derived production of NO has been suggested as an independent predictor of the acute coronary syndrome in patients with coronary disease [6]. Furthermore, endothelial prostacyclin production, largely dependent on COX-2, likely acts synergistically with NO to prevent platelet activation [15].

Endothelial Repair – Circulating Endothelial Progenitor Cells

In 1997, Asahara et al. reported the isolation of putative endothelial progenitor cells (EPCs) from human peripheral blood [1]. Subsequently, blood-derived EPCs were shown to contribute importantly to endothelial repair [20] and ischemia-induced myocardial and peripheral neovascularization [10], and improved endothelial function [22].

Furthermore, several studies have demonstrated an impairment of bone marrow-derived EPC numbers and function in patients with cardiovascular risk factors as compared to healthy subjects, sug-

gesting that cardiovascular risk factors impair the endogenous endothelial repair capacity. Moreover, reduced levels of circulating EPCs were closely associated with an increased risk of cardiovascular events in patients with coronary disease [19, 23].

At present, however, assessment of EPCs is not yet ready for clinical practical use for cardiovascular risk prediction, since the measurements are expensive and time-consuming, need to be further standardized and simplified, and more prospective evidence of an additional impact on cardiovascular risk prediction is required. Currently, EPCs are assessed in the research setting, where they provide important novel insights into the pathophysiology of vascular disease.

Methods for Assessment of "Endothelial Function"

Given the above observations, there has been an intense interest in measurement of endothelial function in numerous clinical and experimental studies. Most studies have used the assessment of endothelium-dependent vasodilation as an index of "endothelial function". This is in part based on the observation that both, endothelium-dependent vasodilation and vasoprotective properties of the endothelium are mediated by NO, at least partly. The measurement of endothelium-dependent vasodilation, in particular in conductance arteries, therefore provides a good estimate of the vascular NO availability.

Several methods to assess endothelium-dependent vasodilation in humans have been developed over the last decades; among them, the most important invasive tests are the evaluation of the change in coronary artery diameter and coronary blood flow after infusion of stimulants of endothelium-dependent vasodilation, such as acetylcholine, during cardiac catheterization. Later, Celermajer et al. have started the assessment of flow-dependent, endothelium-mediated vasodilation by vascular ultrasound [2], a method that has since been widely used, because it is noninvasive, repeatable, reproducible and standardized among well-experienced laboratories. Importantly, measurement of flow-dependent vasodilation has also been shown to represent a good marker of vascular NO availability. This method is based on the increase in laminar shear stress by increased blood flow during reactive hyperemia, that results in a rapid activation of eNOS leading to vasodilation.

Endothelial Function Assessment and Prognosis

Given the important role of endothelial alterations in the development of vascular disease, numerous groups have studied the question of whether the degree of endothelial dysfunction, as measured by impaired endothelium-dependent vasodilation of the coronary artery or the forearm circulation, is related to the risk of cardiovascular events in retrospective and some prospective studies. In all these studies, the degree of endothelial dysfunction was related to the risk of clinical cardiovascular events in the follow-up [12]. Lerman & Zeiher have further reported a multivariant meta-analysis of studies analyzing the association between coronary or peripheral endothelial dysfunction and cardiovascular events (ten studies were included with a total of 2,500 subjects, 1–92 months of follow-up) [13], strongly supporting the concept that endothelial dysfunction is also independently associated with the risk of major cardiovascular events. Moreover, it has been shown that a higher degree of improvement of endothelial function after antihypertensive therapy was associated with a more favorable prognosis in postmenopausal women [16].

On this basis, endothelial function assessment could be reasonably supposed as an emerging adjuvant test for the evaluation of "vulnerable" patients at high risk for future cardiovascular events. However, before endothelial function measurements can be recommended in clinical practice, the ongoing prospective studies analyzing the additive value of standardized endothelial function measurements to careful cardiovascular risk prediction based on classic cardiovascular risk factors, such as the PREVENT-it study, should be awaited. Furthermore, the current methods for the assessment of endothelial function are time-consuming and require a highly qualified and experienced investigator to obtain reproducible results. Therefore, easier to handle systems, such as the Endo-PAT, are currently assessed in large clinical studies for their potential of additive cardiovascular risk prediction.

Conclusion

Endothelial dysfunction represents an early event in the development of atherosclerosis, and is induced by all known cardiovascular risk factors. Furthermore, numerous clinical studies have demonstrated a close relationship of the degree of endothelial dysfunction and the risk of cardiovascular events in the follow-up, suggesting that impaired endothelial function is relevant also to the later stages of atherosclerotic vascular disease precipitating clinical events. Whether endothelial function measurements can be used to improve cardiovascular risk prediction on top of a careful evaluation of classic cardiovascular risk factors is currently studied in large prospective clinical studies.

At present, endothelial function measurements, i.e., mostly the assessment of endothelium-dependent

vasodilation, is a highly valuable research tool to study mechanisms of vascular disease and to assess the impact of therapeutic interventions on vascular function. These measurements can provide important information aiding in designing clinical outcome trials, that then ultimately prove the impact of therapeutic interventions on cardiovascular prognosis.

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