Review

Nitric oxide and coronary endothelial dysfunction in humans

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1. Introduction

The endothelium provides a variety of important functions involved in the cardiovascular homeostasis. The assessment of endothelial function in humans has focused primarily on endothelial dependent vasomotion in response to the release of NO. In particular, clinical studies have evaluated vasomotor tone following changes in flow or stimuli for the release of NO from the endothelium, such as acetylcholine, substance P or serotonin [1]. However, NO not only acts as a vasodilating substance but also affects other functions of the endothelium such as the adhesive properties of the endothelium with respect to the interaction with leucocytes and platelets [2]. Despite the pivotal contribution of in vitro studies for the elucidation of underlying mechanisms, the clinical implication of endothelial function can only be appreciated by studies in humans in vivo, since tissue or cells are exposed to an artificial environment i.e. lacking the neurohumoral influences present in vivo. To critically review the role of NO in coronary endothelial dysfunction in humans the present review focuses on observations made by the in vivo assessment of endothelial dependent vascular responses.

2. Normal endothelial function

In normal individuals increases in blood flow is associated with flow-dependent, endothelium-mediated vasodilation [3] which is related to the release of NO from the endothelium [4,5]. Similarly, acetylcholine, bradykinin or substance P elicit endothelium-dependent dilation of large and small vessels, which, in part, is due to the stimulated release of NO. However, the effect of acetylcholine, the most commonly used agent in the clinical setting, is composed of two divergent actions, that is, vasodilation by the stimulated release of NO and other vasodilating substances such as EDHF from the endothelium, and direct vasoconstricting effects on the underlying vascular smooth muscle, particularly with higher doses. In contrast to acetylcholine, bradykinin—and possibly substance P—is likely to play a physiological role. Tissue bradykinin is inactivated by the endothelial angiotensin-converting enzyme [6] and can be released by increased flow [7]. Endogenous bradykinin appears to be involved in the regulation of basal and flow-mediated coronary vasomotor tone in humans [8]. However the vasodilator effect of bradykinin involves not only NO but also PGI, and EDHF. Consistent with these observations clinical studies have shown that short and long-term ACE-inhibition enhances endothelium-dependent relaxation both in the peripheral and coronary circulation [9–11]. There is evidence that NO is constantly released from the endothelium in humans [12]. In fact Quyyumi et al. have shown that NO contributes to resting epicardial and coronary microvascular tone. Despite the absence of angiographic evidence of arteriosclerosis, the presence of coronary risk factors is associated with reduced resting and stimulated bioavailability of NO from the human coronary circulation [13]. The basal release of NO keeps the vasculature in a ‘dilated state’ and counteracting the vasoconstrictor forces such as noradrenalin, angiotensin or endothelin.

3. Endothelial function in hypercholesterolemia and arteriosclerosis

The endothelial vasodilator function is impaired in the early stages of atherosclerosis. For example, impaired endothelial dependent vasodilation has been noted in large conduit and small resistance vessels in patients with hypercholesterolemia without evidence of arteriosclerotic lesions, as assessed by angiography or intravascular ultra-
sound [14,15]. These observations are consistent with experimental data suggesting that endothelial dysfunction precedes overt atherosclerosis in experimental models of hypercholesterolemia and may represent an important early event predisposing conduit vessels to vasoconstriction.

Assessment of endothelial vasodilator function at different stages of coronary artery disease indicated that a progressive deterioration of endothelial function emerges, including early impairment of acetylcholine-induced vasodilator responses followed by reduced flow-dependent vasodilation [16]. Studies in patients with hypercholesterolemia type IIa, moderately elevated serum cholesterol levels and angiographically smooth coronary arteries revealed a significant endothelial dysfunction of large coronary arteries and coronary microcirculation [14]. In patients with hypercholesterolemia, even angiographically smooth coronary arteries show a striking vasoconstriction in response to intracoronary infusion of acetylcholine. Although no coronary lesions can be detected by angiographic standards in some of those patients it is conceivable that intimal hyperplasia and structural alterations of the vascular wall might have been present. Patients with hypercholesterolemia and normal coronary arteries often have focal intimal thickening and structural remodelling of the vascular wall [17]. Whether or not the basal release of NO is affected remains controversial: while some studies have indicated a preserved response to \( L \)-NMMA in the peripheral vasculature of hypercholesterolemic patients as compared to normal individuals in vitro studies have suggested that basal release of NO is impaired in coronary artery disease [18]. Impaired endothelial vasodilator function is reversible, i.e. by short-term administration of \( L \)-arginine, the precursor of NO [19,20] or following removal of LDL-cholesterol (see below).

4. Endothelial function in the coronary microcirculation

Impaired endothelium-dependent relaxation is not confined to epicardial coronary arteries but emerges in the coronary microcirculation particularly in hypercholesterolemic subjects [14,16] while endothelium-independent vasodilators exert maximal increases in coronary blood flow in hypercholesterolemia. There is experimental evidence that under these circumstances, flow-dependent dilation of the coronary microvasculature is abolished [21]. Since this mechanism of dilation of resistance vessels may be involved in the increased flow, i.e. during states of increased flow such as reactive hyperemia, the impaired endothelial function of the coronary microvasculature in humans may have important implications for subjects with hypercholesterolemia. Moreover, the endothelial modulation of factors such as sympathetic stimulation or platelets and its releasing substances (thromboxane and serotonin) is altered. With normal endothelial function, the release of NO may be associated with vasodilation (counterbalancing the direct vasoconstricting effects of serotonin and thromboxan), however, in the face of a defective endothelial mediated vasodilation, inappropriate coronary vasoconstriction may emerge promoting platelet activation and aggregation.

In general, the degree of endothelial dysfunction of coronary microvasculature correlates with total serum cholesterol levels [22]. However, if the analysis is confined to patients with hypercholesterolemia (rather than to patients with a wide range of serum cholesterol levels) the ratio of LDL/HDL appears to correlate more closely than total serum cholesterol levels with endothelium-dependent dilation in the coronary circulation [23]. Based on the presumed functions of HDL and their relationship to the efficiency of reverse cholesterol transport in subjects with normal and increased LDL levels (for review see [24]), serum levels of HDL cholesterol may be an important determinant for endothelial function and exert protective effects on coronary endothelial function in patients with hypercholesterolemia. Similarly, oxidized LDL or the ability to oxidize LDL (as measured by lagtime) appears to be more closely related to endothelium-dependent dilation in resistance arteries and coronary conduit arteries respectively [25,26]. Preliminary observations suggest that severe endothelial dysfunction as determined by acetylcholine-induced coronary blood flow responses are associated with increased risk for future coronary events [27].

5. Potential mechanisms for the development of endothelial dysfunction

The underlying mechanisms by which risk factors such as hypercholesterolemia or hypertension impair endothelium-dependent relaxation remain to be elucidated. Several possible mechanisms were proposed including: (a) reduced synthesis of NO (b) altered membrane receptor coupling mechanisms affecting the release of NO and (c) impaired diffusion or augmented destruction of NO in the vessel wall. Native LDL lipoprotein has been shown to increase endothelial cell NO synthase generation of superoxide anion [28]. The inhibitory effect of oxidized LDL (presumed to be accumulated in the vascular wall of patients with hypercholesterolemia) may be derived from its ability to interfere with the receptor-operated release of \( L \)-arginine from intracellular stores or the synthesis of the amino acid [29]. Alternatively, high-affinity arginine transport into endothelial cells may be impaired by lysoosphatidylcholine, a component of oxidized LDL [30,31]; in fact a caveolar complex exists between the cationic acid transporter 1, endothelial NO synthase and caveolin [32]. In addition, hypercholesterolemia in patients is associated
with increased plasma and tissue levels of asymmetrical dimethyl-L-arginine (ADMA), inhibiting the NO synthase to generate NO [33]. Thus, in hypercholesterolemia, the intracellular availability of L-arginine may become a rate-limiting factor for the production of NO. Conceivably, hypercholesterolemia may alter the intracellular pathway for L-arginine or affect the stimulated release of NO. Hypercholesterolemia may also affect the NO synthase to produce superoxide radicals which in turn would be able to inactivate NO [28]. The improvement of endothelium-dependent dilation within the human coronary circulation following the administration of L-arginine would be consistent with the hypothesis that by providing the substrate for synthesis of NO, an enhanced formation of NO emerges restoring endothelium-dependent dilation. Notably, L-arginine augmented endothelium-dependent dilation in the coronary microcirculation of patients with hypercholesterolemia but not in control subjects.

6. Functional consequences of impaired NO availability in coronary arteries

The loss of flow-dependent dilation in atherosclerotic segments may favor vasoconstriction at sites where vasodilation could ordinarily occur upon an increase in flow. Such an impaired response to increased flow may in part explain the abnormal vasomotor responses to physiological stimuli in daily life such as exercise, mental stress or exposure to cold environment. Under normal conditions, flow-mediated dilation represents the major mechanism by which the epicardial vessels respond to stimuli, e.g., exercise, that increase myocardial work and oxygen demand. NO mediated, shear-induced dilation opposes the myogenic vasoconstriction elicited by increases in pressure [34,35]. Endothelial modulation of factors such as sympathetic stimulation or platelets and its releasing substances (thromboxane and serotonin) is altered. In the face of endothelial dysfunction, the vessel is more sensitive to the constrictor effects of norepinephrine. Indeed, Vita et al. have shown that patients with evidence of coronary endothelial dysfunction as assessed by acetylcholine, intracoronary infusion of phenylephrine was associated with increased coronary vasoconstriction as compared to normal individuals [36]. Similarly, exercise or exposure to cold activates the sympathetic nervous system and increases circulating levels of catecholamines. Sympathetic activation is also accompanied by complex reflex mechanisms including increases in heart rate and blood pressure thereby enhancing myocardial oxygen demand. In patients with normal coronary arteries exercise and exposure to cold produce dilation of epicardial coronary arteries [37]. In contrast, patients with arteriosclerotic coronary arteries demonstrate ‘paradoxical’ epicardial coronary vasoconstriction in response to exercise, cold pressure test or mental stress [38-40] despite similar effects on central hemodynamics or plasma levels of catecholamines. In such patients there is a close relationship between endothelial function and response of the identical coronary segment to the cold pressure test. Coronary vasoconstriction is usually observed only in segments with acetylcholine-induced epicardial coronary vasoconstriction (indicating endothelial dysfunction). Moreover, sympathetic activation is an important trigger mechanism of myocardial ischemia in patients with coronary artery disease and stable angina. There is evidence that exercise induces dynamic vasoconstriction at the site of arteriosclerotic lesions [40].

Conceivably, inappropriate vasoconstriction due to endothelial vasodilator dysfunction of atherosclerotic epicardial arteries may play an important role in triggering myocardial ischemia in patients with (intermediate) coronary stenoses. Even though the extent of coronary vasoconstriction elicited by sympathetic activation is usually less than 25 to 30%, such an increase in arterial tone may be sufficient to convert a non-flow-limiting into a critical stenosis with ensuing decreases in blood flow; i.e., in intermediate coronary lesions of 50-70%. Thus, in coronary lesions with stenoses between 50 and 70% (luminal diameter) an abnormal vasoconstrictor response due to the loss of endothelium-mediated vasodilation may account for the mismatch between myocardial oxygen supply and demand, with other words, a link may exist between endothelial vasodilator dysfunction at the site of the stenosis and myocardial ischemia and explain episodes of angina associated with daily life activities.

Notably, coronary vasomotor tone may be further enhanced by humoral factors released from activated platelets during ischemia, plaque rupture or simply during circadian peaks of platelet aggregability. A recent study has shown that intracoronary infusion of serotonin resulting in concentration equivalent to those during platelet activation caused severe epicardial coronary vasoconstriction in patients with coronary artery disease and endothelial dysfunction whereas coronary diameters increased in normal subjects [41,42]. Thus, the vasomotor response to serotonin is dependent on the integrity of the endothelium. Platelet-mediated vasoconstriction may only occur if the endothelium is dysfunctional. Importantly, the intact endothelium plays an important role in maintaining the balance between proaggregatory and antiaggregatory behaviour of platelets since both NO and prostaglandins inhibit platelet aggregation and adhesion. Hence, defective endothelial function predisposes to the deposition of platelets. Indeed, increased platelets adhesion has been demonstrated in human arteriosclerotic arteries. Vice versa, stimulation of NO release from the vascular endothelium promotes inhibition of platelet aggregation in the human circulation and this inhibitory effect is attenuated in patients with atherosclerosis [43].
Taken together, with normal endothelial function, the release of NO may be associated with vasodilation (countering the direct vasoconstricting effects of serotonin and thromboxan), however, in the face of a defective endothelial mediated vasodilation, inappropriate coronary vasoconstriction may emerge promoting platelet activation and aggregation [44].

Since experimental data suggest that the endothelium plays an important role in maintaining vascular structure [45], the impaired basal release of atherosclerotic coronary arteries as demonstrated in vitro may have long-term implications concerning vascular remodelling of coronary arteries. Since NO interferes with the action and synthesis of endothelin, the lack of NO activity may favor the vascular expression of endothelin. Indeed, increased circulating and tissue endothelin immunoactivity has been observed in patients with coronary artery disease [46]. In fact, the expression of endothelin in the coronary circulation is increased in humans with coronary endothelial dysfunction [47].

8. Effect of cholesterol lowering by statins on endothelial dysfunction

Several longterm studies have applied lipid lowering drugs for several months and evaluated endothelial function before and after this intervention in patients with coronary artery disease and hypercholesterolemia. Leung et al. [52] were the first to assess endothelium-dependent and -independent dilation of coronary epicardial arteries before and 6 months following a lipid-lowering regimen, consisting of dietary restrictions and oral treatment with cholestryramin. A decrease in plasma cholesterol of approximately 30% was achieved at follow-up, in comparison to baseline. Before treatment, intracoronary coronary infusion of acetylcholine causes severe vasoconstriction of epicardial arteries, whereas a moderate vasodilation was observed at follow-up. Intracoronary infusion of nitroglycerine elicited a similar degree of vasodilation at baseline and follow-up. Egashira et al. studied a small group of nine hypercholesterolemia patients before and after treatment with pravastatin, an inhibitor of HMG-CoA reductase [53]. The acetylcholine-induced vasoconstriction of the epicardial artery was less and the the acetylcholine-induced increases in coronary blood flow were greater after pravastatin. In a normocholesterolemic control group the effects of acetylcholine were similar before and after 8 months. Thus, the lipid lowering intervention (272 to 187 mg/dl) was associated with an improvement of endothelial function of coronary conduit and resistance vessels. Two controlled trials have yielded similar beneficial effects of HMG-CoA reductase inhibitors on endothelial function in the coronary circulation [54,55]. In one of the latter trials, the incidence of angina was reduced [55]. Moreover, short-term (3 month) cholesterol lowering decreases the size and severity of perfusion abnormalities as assessed by positron emission tomography after dipyradomole in hypercholesterolemic patients with coronary artery disease [56], myocardial ischemia, angina pectoris [57–59] and improves coronary flow [60]. The rapid improvement in myocardial perfusion is unlikely to be attributed to regression of epicardial coronary lesions. In fact, seminal studies by Brown et al. have clearly shown that the extent of lesion regression is minimal and if it occurs takes years to be measureable [61]. Conceivably, improvement of endothelial function has contributed to the improved coronary perfusion following cholesterol lowering with statins [60]. The improvement in endothelial function may not only be related to reduction of LDL-cholesterol but to a specific upregulation of the synthesis of NO synthase by statins which increase the mRNA for the endothelial NO synthase [62]. In addition, prolonged treatment with statins may reduce vascular radical formation. Interestingly, longterm therapy with probucol over 1 year combined with lovastatin was more effective than lovastain given alone in improving endothelial dysfunction of epicardial coronary...
arteries [55]. Since probucol acts as an antioxidant with minor effects on lipid plasma levels, these clinical observations are consistent with experimental data suggesting that inactivation of NO may play a prominent role in hypercholesterolemia, diabetes and arteriosclerosis.

9. Inactivation of NO as a cause of endothelial dysfunction

Hypercholesterolemia, diabetes mellitus or heart failure are all associated with increased oxidative stress, either due to increased radical formation and/or decreased radical scavenging enzyme activity (see Fig. 1). Superoxide anions production may be enhanced by stimulation of the NADPH oxidase in response to angiotensin II [63,64]. Antioxidants such as vitamin C or E, PEG-SOD or probucol have been applied clinically to test the concept that inactivation of NO may play a prominent role in cardiovascular disorders. In fact, the water soluble antioxidant vitamin C has been shown to improve endothelium-dependent dilation (and availability of NO) in patients with hypercholesterolemia, hypertension, diabetes or coronary artery disease. Importantly, vitamin C improves endothelium-dependent vasomotor capacity after acute administration [65] and improves myocardial perfusion during long-term therapy [66], again implying that increased oxidative stress contributes to endothelial dysfunction in hypertensive patients with coronary artery disease. Moreover, evidence has been presented that a weak glutathione related enzymatic antioxidant is present in human atherosclerotic lesions [67], while intracoronary infusion of reduced glutathione improves endothelial vasomotor response to acetylcholine in the human coronary circulation [68]. Furthermore L-2 oxothiazolidine-4-carboxylic acid, which augments intracellular glutathione, improves endothelial-dependent relaxation in patients with coronary artery disease [69]. The impressive and instant improvement of NO availability following administration of antioxidants supports the role of oxygen species in the impaired endothelial dependent relaxation in coronary artery disease and its risk factors and are consistent with the notion, that the cellular redox state may be an important regulator of endothelium-derived NO. Interestingly, low plasma ascorbic acid independently predicts the presence of an unstable coronary syndrome [70] which would support the role of impaired endothelial dysfunction in the clinical setting of angina. Moreover, the activity of superoxide dismutase in the extracellular space (EC SOD) or in vascular smooth muscle cells (Cu/Zn SOD) may play an important role in keeping the balance between radical formation and scavenging.

Vitamin E is thought to protect LDL from oxidation within the vascular wall. However, the clinical observations with vitamin E on the effect on endothelium-dependent relaxation are conflicting. In our experience the beneficial effect of longterm vitamin E treatment on endothelium-dependent dilatation is confined to patients with increased levels of autoantibodies against oxidized LDL suggesting that vitamin E works only in those patients whose endogenous oxygen stress defence mechanisms cannot cope with the generation of oxygen radicals produced [71].

10. Peripheral endothelial function as a surrogate for the coronary circulation

The assessment of endothelial function in the coronary circulation can only be achieved by cardiac catheterization and interventional techniques including Doppler flow measurements. In the last 7–8 years several investigators have used a non-invasive approach, introduced by Deanfield group, to identify patients at risk by determining endothelial function in peripheral arteries, mostly in the brachial artery. Whether or not the evaluation of endothelial function in the forearm represents a useful surrogate to predict the development of atherosclerosis in humans (i.e. for the clinically more important coronary and cerebral circulation) is an open but a relevant question, given the limitations to evaluate the latter circulatory beds. A comparison of flow-dependent dilation in the brachial artery and acetylcholine-induced coronary vascular response demonstrated a reasonable relationship, although different stimuli for release of NO were used [72]. However, the sensitivity for detecting coronary endothelial dysfunction was only 49% in that study [72]. It is unclear whether or not determination of the same stimuli in the brachial and coronary artery would provide better sensitivity. However, an even more important issue is whether endothelial dysfunction of the brachial artery predicts risks for future coronary events (rather than simply the presence of coronary arteriosclerosis). Large-scale clinical studies are needed to address this important clinical question.

Fig. 1. Balance between radical formation and radical scavenging is dependent on the activity of enzymes systems generating superoxide anions in the vascular wall such as the NADPH oxidase, and radical scavenging enzymes such as the different isofroms of superoxide dismutases. There is evidence that the NADPH oxidase is activated by angiotensin and may exceed the capacity of SODs to scavenge radicals resulting in increased oxidative stress and inactivation of NO.
11. Conclusions

In summary clinical studies have been able to evaluate endothelial-dependent vasomotor capacity in the coronary circulation in patients with risk factors and/or evidence of coronary artery disease. There is clear evidence that endothelial-dependent vasodilation is impaired in coronary artery disease and this functional alteration is associated with impaired myocardial perfusion and ischemia. Thus, improvement of endothelial vasodilator capacity is a clinically relevant target for therapy and may prevent clinical symptoms of ischemia. Whether or not prevention of endothelial function has prognostic implications is an important question and remains to be determined. Several interventions have been effective in restoring endothelial vasodilator responses such as lipid lowering, antioxidants or ACE-inhibitors. Further studies should be able to elucidate whether the overall clinically beneficial effects of these interventions are related to improvement of endothelial function which includes much more then vasodilator capacity, i.e. attenuation of leucocyte adhesion, prevention of platelet aggregation or favoring profibrinolytic activity.

References


