Endothelial Dysfunction

More than a quarter of a century ago Robert Furchgoltt demonstrated that removing the endothelial layer from the isolated aorta of a rabbit prevents normal relaxation in response to acetylcholine. The ability of the endothelium to elicit relaxation was soon extended to more relevant physiological stimuli (adenosine triphosphate, bradykinin and serotonin). The endothelial cells cause arterial relaxation by releasing a powerful vasoactive substance(s) that diffuses to the underlying layers of vascular smooth muscle and thus was termed endothelium-derived relaxing factor (EDRF). EDRF stimulates the activity of soluble guanylyl cyclase with the subsequent production of cyclic guanosine monophosphate (cyclic GMP) in the vascular smooth-muscle cells; it is also avidly scavenged by superoxide anions. These findings led Robert Furchgott and Louis Ignarro to the proposal that EDRF is nitric oxide (NO), a hypothesis that was proved correct by Salvador Moncada. Further investigations have identified many components of the pathway producing NO, from the stimulation of muscarinic (and other) receptors on the endothelial cells to the eventual activation of an enzyme, endothelial NO synthase (eNOS or NOS3), that transforms L-arginine into NO. When inhibitors of the enzymes involved in the pathway became available, it soon appeared that NO was termed endothelium-derived relaxing factor (EDRF). EDRF stimulates the activity of soluble guanylyl cyclase with the subsequent production of cyclic guanosine monophosphate (cyclic GMP) in the vascular smooth-muscle cells; it is also avidly scavenged by superoxide anions. These findings led Robert Furchgott and Louis Ignarro to the proposal that EDRF is nitric oxide (NO), a hypothesis that was proved correct by Salvador Moncada. Further investigations have identified many components of the pathway producing NO, from the stimulation of muscarinic (and other) receptors on the endothelial cells to the eventual activation of an enzyme, endothelial NO synthase (eNOS or NOS3), that transforms L-arginine into NO. When inhibitors of the enzymes involved in the pathway became available, it soon appeared that NO was involved in many aspects of biology. However, in the years that followed it also became evident that endothelial cells can affect the tone of the underlying smooth muscle in more than one way. This article focuses on NO because its reduced production characterises endothelial dysfunction, which is the first step (at least in the author’s mind) in the long chain of events that leads to atherosclerosis and coronary disease.

The Protective Role of the Endothelium

The release of NO plays an essential role in the protection exerted by the endothelial cells against coronary disease. Indeed, the endothelial mediator not only prevents abnormal constrictions (vasospasm) of the coronary arteries, but also inhibits the aggregation of platelets, the expression of adhesion molecules at the surface of the endothelial cells and, hence, the adhesion and penetration of white blood cells (macrophages) and the release and action of the vasoconstrictor and mitogenic peptide endothelin-1 (see Figure 1). The protective release of NO is exacerbated by the local presence of factors involved in the coagulation of the blood, in particular the formation of thrombin and the aggregation of platelets. If this protective role of NO fails, the stage is set for the inflammatory response that will eventually lead to the formation of atherosclerotic plaques.

The protective role played by the endothelial cells against unwanted coagulation has been demonstrated repeatedly not only in vitro but also in vivo. If constricted isolated animal or human coronary arteries with healthy endothelium are exposed to thrombin or aggregating platelets, they relax immediately because of the endothelial release of NO. If the endothelium has been removed, this relaxation is no longer observed, and the aggregating platelets induce vigorous constrictions as a result of their liberation of thromboxane A2 and serotonin (5-hydroxytryptamine). When aggregating human platelets were added to the solution, vasospasm occurred in the arteries without endothelium. Thus, the healthy endothelium appears to protect blood vessels from vasospasm when they are threatened by aggregating platelets, thrombin or the arrival of a thrombus.

The two most important substances released by aggregating platelets that trigger the activation are serotonin and adenosine diphosphate (ADP). The former is dominant and stimulates 5-HT1 receptor agonist that acts on P2Y purinergic receptors. These two products trigger distinct signalling cascades in the endothelial cells (see Figure 1). The stimulation of serotonergic receptors (and of those for thrombin) is coupled to the activation of eNOS through pertussis toxin-sensitive G-proteins, and purinergic receptors follow a Gq-dependent cascade.

If platelet aggregation were to occur in a coronary artery with a normal endothelium, the release of serotonin (and ADP) and the local initiation of the coagulation cascade (with the production of thrombin) would become a strong signal for the endothelial cells to release NO. The endothelial mediator will not only cause the underlying smooth-muscle cells to relax, allowing the beginning aggregate to be flushed away by the flow of blood, but will also exert, hand in hand with prostacyclin, which is also released, an immediate feedback inhibition of the platelet aggregation process. Under normal conditions, if the endothelial barrier is interrupted by injury, the aggregating platelets can move to the immediate vicinity of the vascular smooth-muscle cells. The thromboxane
A<sub>2</sub> and serotonin that they release cause constriction, which initiates the vascular phase of haemostasis. Such endothelium-dependent responses to platelets are not present equally in all blood vessels, but are most prominent in the coronary and cerebral circulations. The ability of endothelial cells to release NO in response to aggregating platelets and other stimuli can be modulated in the intact organism by a number of chronic factors in both positive and negative ways. Their inability to do so sufficiently has become a hallmark and indeed a predictor of cardiovascular disease.

Positive Modulation of Endothelial Responsiveness

Both acute and chronic increases in flow and the resulting increasing force of shearing (shear stress) of the blood on the endothelial cells augment the release of EDRF. In the coronary circulation the effect of shear stress involves the local production of the autacoid bradykinin, which stimulates the release of NO through a Gq-dependent mechanism. The chronic effect of shear stress involves an upregulation of eNOS in the endothelial cells, leading to a greater release of NO. This then explains the repeatedly demonstrated beneficial effects of regular exercise on endothelial function.

In ovariectomised animals the reintroduction of physiological levels of 17-β-estradiol potentiates endothelium-dependent relaxation of the isolated arteries. The potentiating effect of oestrogens on the release of NO has been confirmed repeatedly and involves both non-genomic and genomic (upregulation of eNOS) effects. In the coronary artery the potentiation is seen only with stimuli that activate G<sub>i</sub>-coupled receptors on the endothelial cells. It is counteracted by progesterone. The potentiating effect of oestrogens on the release of NO by the endothelium helps to explain why women are protected against coronary disease, at least until the age of menopause. The opposing effects of oestrogens and progesterone on endothelial function may help to explain why hormone replacement therapy has not always had the expected beneficial effect on the occurrence of cardiovascular events.

Dietary factors also affect the ability of endothelial cells to release NO. Thus, the chronic intake of ω-3-ununsaturated fatty acids augments the endothelium-dependent relaxation of isolated coronary arteries to aggregating platelets and other stimuli. This augmentation of endothelial function is also observed in the human circulation. The same holds true for the intake of polyphenols, whether present in red wine, green tea or dark chocolate.

Negative Modulation of Endothelial Responsiveness

Unfortunately, with increasing age the endothelium has a reduced capacity to release NO, as has been demonstrated repeatedly both in animals and in people. There is an overwhelming amount of data demonstrating that smoking, both chronically and acutely, inhibits the expression of eNOS and thus the ability of the endothelium to release NO. More recently, it also became obvious that chronic exposure to polluted air and intermittent hypoxia (obstructive sleep apnoea) reduce endothelium-dependent responsiveness.

Dietary factors can have a negative impact on endothelial function. Thus, in animals a chronic hypercholesterolaemic diet blunts endothelium-dependent responsiveness. In humans hypercholesterolaemia is associated with endothelial dysfunction, and normalisation of the cholesterol level (e.g. following treatment with statins) in the blood restores the response. These observations are in line with the studies demonstrating that obese humans exhibit reduced responses to endothelium-dependent vasodilators.

Diseases that constitute major risk factors of coronary disease are also accompanied by an abnormal function of the endothelium. Thus, diabetes has long been associated with impaired arterial endothelium-dependent relaxation. Likewise, the endothelium-dependent relaxation is reduced in isolated arteries from different animal models of hypertension, as is the response of endothelium-dependent vasodilators in hypertensive humans. However, both in diabetes and in hypertension, the reduced endothelium-dependent relaxation/vasodilatations are not due solely to a diminished release of NO but also to the augmented production by the endothelial cells of vasoconstrictor prostanoids.

Regenerated Endothelial Cells

Under normal conditions mature endothelial cells remain quiescent for many years because they are in mutual physical contact (contact...
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inhibition). However, their turnover is accelerated by the cardiovascular risk factors mentioned previously. Eventually, these cells undergo apoptosis, are removed by circulating blood and are replaced rapidly by regenerated endothelial cells. It is still uncertain what the exact contribution in this regeneration process is of neighbouring cells freed of the contact inhibition and circulating endothelial progenitor cells.74-78

Whatever their origin, regenerated endothelial cells appear to be dysfunctional. This conclusion is based on experiments performed in porcine coronary arteries. In this preparation, one month after in vivo balloon denudation of the endothelium of part of the artery a total relining of the endothelial surface occurred. However, rings with endothelium of the previously denuded part of the artery did not fully relax to aggregating platelets, serotonin or thrombin, and the remaining relaxation was no longer inhibited by pertussis toxin, suggesting that Gi-protein-mediated responses are defective in regenerated endothelial cells.79,80,81 In contrast, relaxation evoked by agonists that employ the Gq-signalling cascade was normal, implying a selective dysfunction of the Gi-dependent responses in regenerated endothelial cells. This selective dysfunction was reduced by the chronic intake of o3-unsaturated fatty acid and acetylated by a chronic hypercholesterolemic diet, which resulted in the occurrence of typical atherosclerotic lesions in the area of previous denudation.44,82 These observations prompted the conclusion (at least by the author and his colleagues) that the dysfunction of regenerated endothelial cells is the first step allowing the atherosclerotic process.

Further work to analyse the molecular mechanisms underlying the dysfunction of regenerated endothelial cells was performed on primary cultures (with all the limitations of cell culture studies, in terms of relevance to the intact organism) derived from either regenerated or native endothelium.78,80-82 The cultures derived from regenerated endothelium had the appearance and markers of senescent cells, had a reduced expression and activity of eNOS, produced more oxygen-derived free radicals, took up more modified low-density lipoprotein cholesterol (LDL) and generated more oxidised LDL (oxLDL). However, the presence of Gi-proteins was comparable in the two cell types. Those phenotypic and functional changes are in line with the genomic changes observed in cultures of regenerated endothelial cells. Moderately increased concentrations of oxLDL reduce the production of EDRF by endothelial cells and inhibit endothelium-dependent relaxations to serotonin.83,84 hence the conclusion that the augmented presence in regenerated endothelial cells of oxLDL is the cause of the selective loss in Gi-protein-mediated responses and the resulting inability to respond to serotonin and thrombin, setting the atherosclerotic process in motion (see Figure 2).

It would be naive to claim that this is the only negative effect of oxLDL that obviously plays a central role in the atherosclerotic process.85-87 It has a direct inhibitory effect on the expression and activity of eNOS.88,89 It also enhances the activity of arginase, which competes with NO for the common substrate arginine.90-92 A greater production of superoxide anions will reduce the bioavailability of NO and increase the levels of peroxynitrite.90-93-95 A number of other genomic factors and endogenous mediators may accelerate or contribute to the atherosclerotic process.96-99 However, the ultimate result is that the endothelial cells cannot produce enough NO in response to platelets and thrombin and allow the inflammatory reaction leading to atherosclerosis.100

Conclusions

Normal endothelial cells respond to aggregating platelets and thrombin by releasing NO. This key endothelial mediator relaxes the underlying vascular smooth-muscle cells and immediately inhibits the platelet aggregation process. It also inhibits the expression of adhesion molecules, and thus the adhesion and penetration of white blood cells. NO prevents the growth and proliferation of vascular smooth-muscle cells, reduces the production and action of endothelin-1 production and limits the oxidation of LDL. Ageing, insults to the coronary endothelial layer (including lifestyle factors such as the Western diet, pollution and smoking) or diseases facilitating cardiovascular events (diabetes and hypertension) create a vicious circle in which damaged endothelial cells undergo apoptosis and new ones are regenerated. However, the function of these regenerated cells differs from that of native endothelial cells, leading to accelerated cell senescence and abnormal production of NO and facilitating the inflammatory reaction leading to atherosclerosis.

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