Regeneration of the endothelium in vascular injury

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Abstract

The endothelium mediates relaxations (dilatations) of the underlying vascular smooth muscle cells. The endothelium-dependent relaxations are due to the release of non-prostanoid vasodilator substances. The best characterized endothelium-derived relaxing factor (EDRF) is nitric oxide (NO). The endothelial cells also release substances (endothelium-derived hyperpolarizing factor, EDHF) that cause hyperpolarization of the cell membrane of the underlying vascular smooth muscle. The release of EDRF from the endothelium can be mediated by both pertussis toxin-sensitive G_i

(alpha$_2$-adrenergic activation, serotonin, thrombin) and insensitive G_q (adenosine diphosphate, bradykinin) coupling proteins. The ability of the endothelial cell to release relaxing factors can be upregulated by impregnation with estrogens, exercise and antioxidants, and down-regulated by oxidative stress and increased presence of oxidized LDL. Following injury or apoptotic death, the endothelium regenerates. However, in regenerated endothelial cells, there is an early selective loss of the pertussis-toxin sensitive mechanisms of EDRF-release. Functional studies suggest that abnormal handling of LDL because of increased oxidative stress play a key role in this selective loss. Genomic analysis demonstrates the emergence of fatty acid binding protein-A (A-FBP) and metalloproteinase-7 (MMP7) in regenerated endothelial cells. The reduced release of NO resulting from the endothelial dysfunction in regenerated areas creates a *locus minoris resistentiae* which favors the occurrence of vasospasm and thrombosis as well as the initiation of atherosclerosis.

Key words

NO, G-proteins, oxLDL, regenerated endothelium

Introduction

Thirty years ago Robert Furchgott demonstrated that the endothelial cell layer of the rabbit aorta mediates relaxation in response to acetylcholine (1). This ability of the endothelium to elicit relaxation of the underlying vascular smooth muscle has been extended to more relevant physiological stimuli [e.g. adenosine di (ADP)- and tri (ATP)-phosphate, thrombin, histamine, bradykinin, serotonin]. The endothelial cells cause relaxation by releasing one or more diffusible vasoactive substances [endothelium-derived relaxing factor (EDRF)]. The original EDRF described by Furchgott is nitric oxide (NO) (2-4). However, endothelial cells can affect the tone of the underlying smooth muscle in more than one way. Thus, besides NO, a number of endothelium-derived factors (EDHF) or the opening of myo-endothelial gap junctions can cause NO-independent hyperpolarizations of the underlying vascular smooth muscle (5-7). In addition, endothelial cells can release endothelium-derived contracting factors, EDCF (2, 8, 9). Endothelial dysfunction occurs when the ability of the endothelial cells to release NO and EDHFs is reduced, that to produce EDCF is augmented. Such dysfunction appears to be an initial step in the chain of events that leads to atherosclerosis and coronary disease (10-13). It

has become a hallmark of cardiovascular disease and a predictor of cardiovascular events.

Under normal conditions, native endothelial cells remain quiescent for many years, as long as they are in mutual physical contact [contact inhibition]. However, with ageing, endothelial turnover begins, and certain endothelial cells undergo apoptosis, detach and are washed away by the circulating blood, a process accelerated by the major cardiovascular risk factors. These cells are replaced rapidly by regenerated endothelial cells, whether produced by the proliferation of neighboring cells freed of the contact inhibition or originating from circulating endothelial progenitor cells. Whatever their origin, regenerated endothelial cells appear to be dysfunctional and this dysfunction favors the atherosclerotic process. This essay summarizes the evidence, mainly from the author's laboratory(ies), which permits the preceding conclusion.

The protective role of the native endothelium

The release of NO plays a pivotal role in the protection exerted by the endothelium against coronary disease. Indeed, NO not only prevents abnormal constrictions (vasospasm), but also inhibits platelet aggregation as well as the expression of adhesion molecules at the surface of the endothelial cells, and hence prevents the adhesion and penetration of white blood cells (macrophages) (12, 13).

The protective release of NO is triggered by the local generation of thrombin and products [5-hydroxytryptemine (serotonin, 5-HT) and adenosine diphosphate (ADP] platelets released by aggregating platelets [Figure 1].

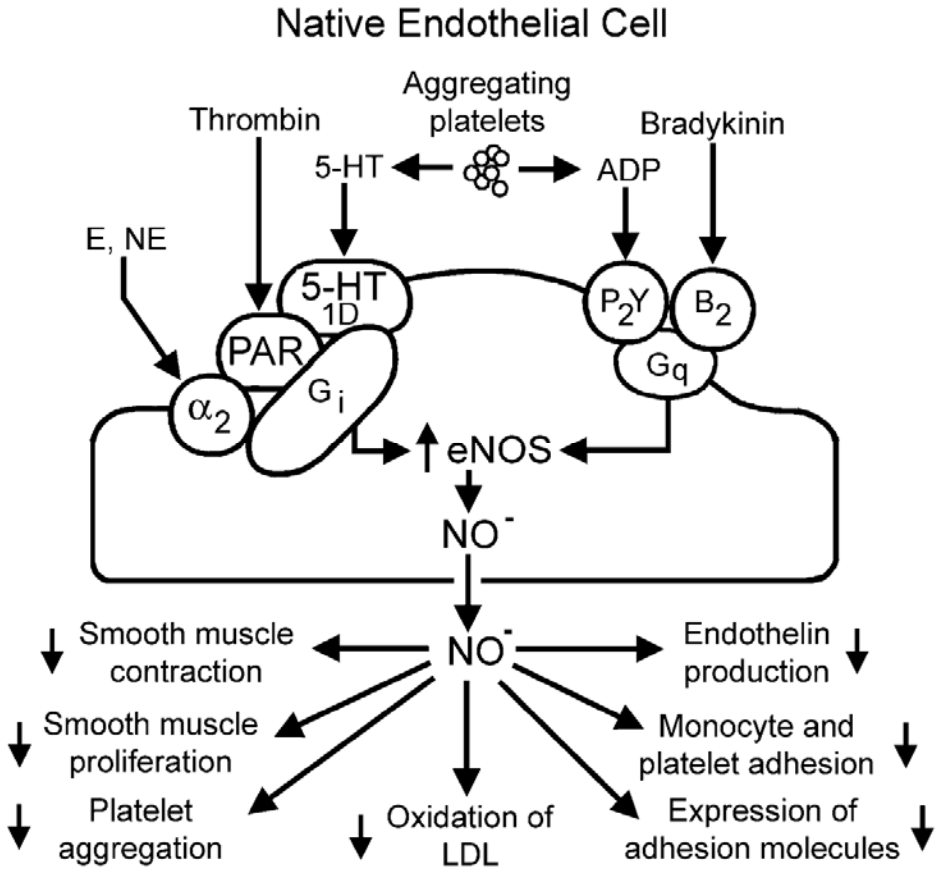

Figure 1

Of the substances released by aggregating platelets that trigger the release of NO, serotonin is the dominant one. The monoamine activates 5-HT_{1D} serotonergic receptors on the endothelial cells which are coupled to endothelial nitric oxide synthase (eNOS) through pertussis toxin-sensitive Gi-proteins. ADP is a lesser

contributor which stimulates endothelial P_{2y} purinergic receptors, coupled to eNOS by Gq- proteins (12-14). If platelet aggregation is initiated in a coronary artery with a normal endothelium the release of serotonin and ADP and the subsequent activation of the coagulation cascade with the formation of thrombin combine to stimulate the endothelial cells to release more NO. NO diffuses to the underlying smooth muscle which relaxes, allowing the beginning aggregate to be flushed away by the flowing blood. It also, in synergy with endothelium-derived prostacyclin, exerts a feed-back inhibition of platelet aggregation. If the endothelial barrier is interrupted by injury, the aggregating platelets approach the vascular smooth muscle cells. They release thromboxane A_2 and serotonin which cause an immediate vasoconstriction, underlying the vascular phase of hemostasis (12,13). The release of NO by the native endothelium in response to aggregating platelets is potentiated by dietary supplementation with ω_3-unsaturated fatty acids (15,16) and impaired by hypercholesterolemia (17-19).

Regenerated endothelial cells

To study the impact of regeneration on endothelial function, a series of studies were conducted in the pig (18, 20-26). Under anaesthesia, a segment of the left anterior descending coronary artery [LAD] was gently denuded of the endothelium. One month after this *in vivo* removal of the endothelium of part of the artery, a total relining of the endothelial surface had occurred. The regenerated

endothelium expressed von Willebrand factor and contained eNOS. However, rings with such regenerated endothelium exhibited a major impairment of the endothelium-dependent relaxation to aggregating platelets, serotonin, ergonovin or thrombin, and a greater propensity to exhibit endothelium-dependent contractions. and the remaining relaxation was no longer inhibited by pertussis toxin. This indicated that Gi-protein coupling is defective in regenerated endothelial cells. By contrast, the Gq-coupling to eNOS appeared intact as relaxations to bradykinin were not impaired. Likewise, endothelium-dependent relaxations evoked by the calcium ionophore A23187 were comparable to those obtained in rings of the same arteries covered with native endothelial cells. Taken in conjunction with the normal response to bradykinin, this demonstrated that the intrinsic ability of the regenerated endothelium to produce NO was not affected. This series of experiments prompted the obvious conclusion that there is a selective loss of the Gi-dependent coupling to eNOS in regenerated endothelium [Figure 2]. This selective dysfunction was still obvious six months after the denudation procedure, indicating that in terms of the protective action of the endothelium, areas with regenerated endothelial cells constitute a permanent *locus minoris resistentiae*. The occurrence of this selective endothelial dysfunction could be prevented in part reduced by chronic intake of ω_3-unsaturated fatty acid, and was exacerbated by hypercholesterolemia. Indeed, the combination of a hypercholesterolemic diet with

the endothelium removal resulted in typical atherosclerotic lesions only in the previously denuded segments. Hence, we concluded that the dysfunction of regenerated endothelial cells is an initial step allowing the inflammatory reaction leading to the formation of atherosclerotic plaques (12, 13). Similar conclusions were reached concerning the endothelial dysfunction following heterotropic heart transplantation, a dysfunctions which was accentuated by injury of the coronary endothelium at implantation (27-29).

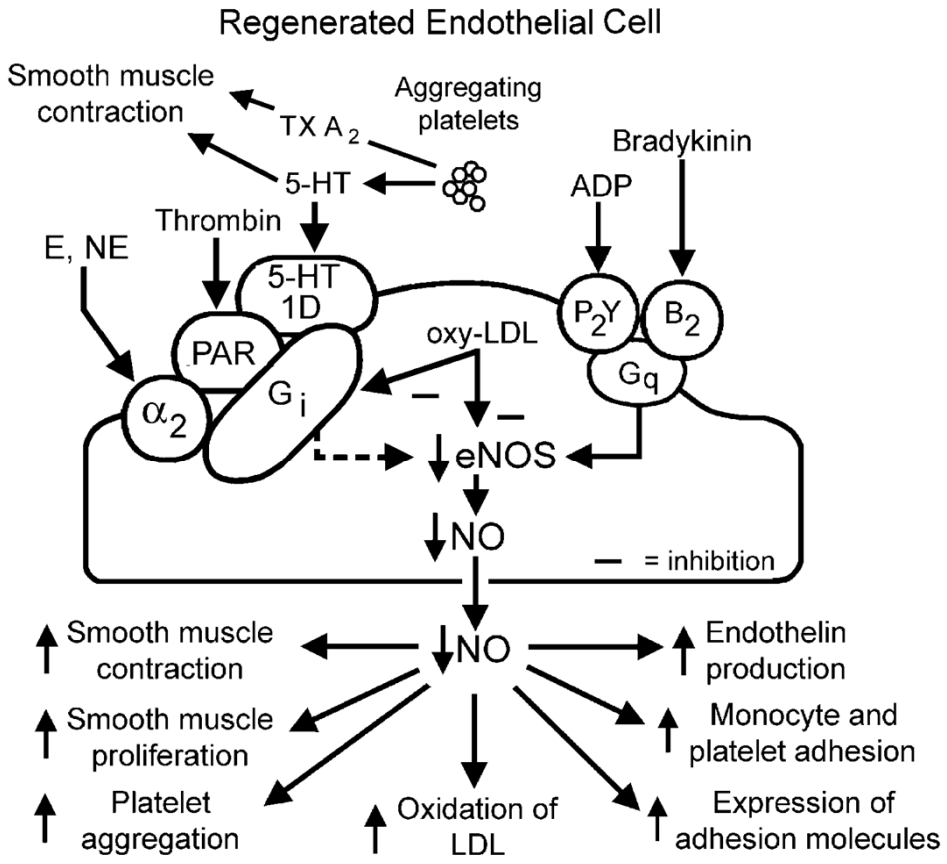

Figure 2

To attempt to unravel the molecular mechanisms underlying the dysfunction of the regenerated endothelium primary cell cultures were grown derived from the endothelium harvested from segments of coronary arteries of the same hart covered with either native or regenerated endothelium (30-34). Compared to the former, those derived from regenerated endothelium exhibited the following phenotypic changes: a]the presence of multiple enlarged cells and cells containing several nuclei, an appearance characteristic of early senescence, as confirmed by the greater presence of β-galactosidase; b] a reduced expression and activity of eNOS; c] a greater production of oxygen-derived free radicals (ROS); d] a greater uptake of modified low-density lipoprotein cholesterol (LDL), a phenomenon confirmed in *ex vivo* measurements in intact coronary arteries lined with regenerated endothelium; e] a greater generation of oxidized LDL (oxLDL); f] a reduced activity of Gi-proteins despite the immunostaining demonstration of their unchanged presence, a phenomenon exacerbated by a hypercholesterolemic diet; and g] an indication of accelerated apoptosis. Increases in the extracellular concentration of oxLDL decrease the release of EDRF by endothelial cells and selectively inhibit endothelium-dependent relaxations evoked by serotonin. Thus it seems logical to conclude that the augmented presence of oxLDL in the regenerated endothelium initiates a selective dysfunction of the Gi-proteins and the resulting inability to respond to stimuli such as serotonin released from aggregating

platelets, curtailing the protective role of the endothelial cells in preventing the atherosclerotic process. Obviously, this is but one of the negative impacts of oxLDL on endothelial function [Figure 3] (12, 13).

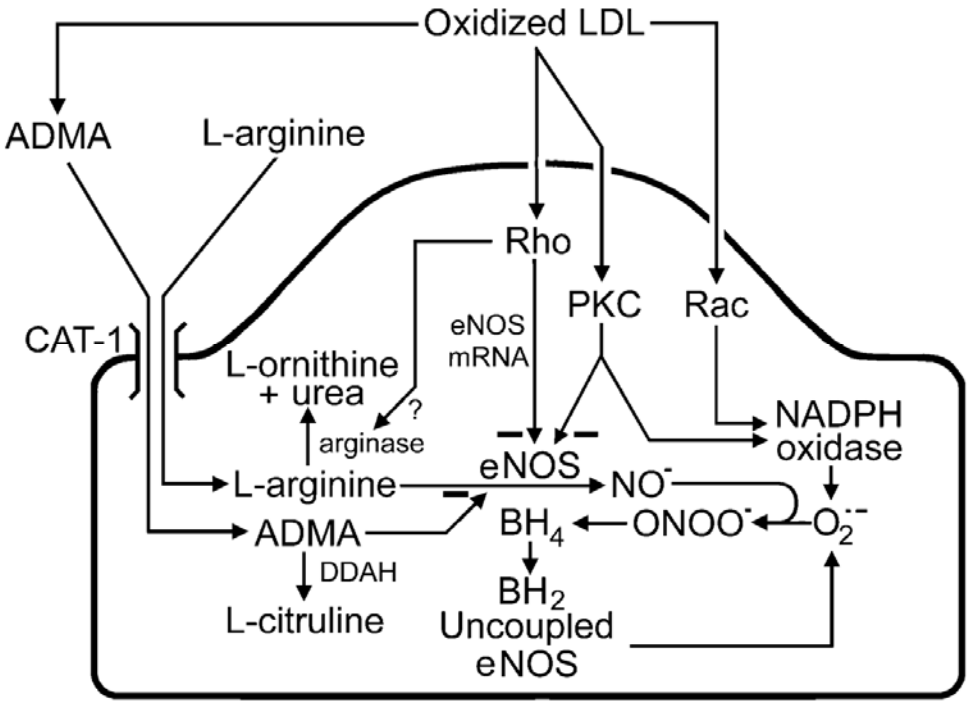

Figure 3

A comparison was performed of the genomic profile of primary cultures of native and regenerated endothelial cells harvested from the same hearts, using a microarray approach (34). As could be anticipated, a large number of genes were expressed differentially in the two cell culture types. The down- or up-regulations of genes leading to the production of proteins involved in vasoconstriction,

coagulation, apoptosis and inflammation were consistent with the phenotypic modifications mentioned above. Pathway analysis indicated the central role of ROS and oxyLDL in the observed changes in genomic expression. The most surprising finding was the emergence, only in cultures of regenerated endothelial cells of the genes for fatty acid binding protein A [A-FABP] and mineraloproteinase-7 [MMP7]. Whereas A-FABP is recognized as a player in the atherosclerotic process 35, 36), MMP7 so far has been mainly involved in inflammatory responses of the airways (37). Although many of the genomic changes observed in primary cultures of regenerated endothelial cells correspond to those obtained in cultures of native endothelium driven to senescence by multiple passaging, they were not identical. In particular, senescent native endothelial cells do not express A-FABP and MMP7, which makes them an inappropriate cell culture model for endothelial regeneration (38).

Conclusions

Native endothelial cells increase their release of NO in response to aggregating platelets (and thrombin). NO in turn relaxes the underlying vascular smooth muscle, inhibits platelet aggregation, and reduces the expression of adhesion molecules, and thus the adhesion and penetration of macrophages. It also inhibits the proliferation of vascular smooth muscle cells, prevents the production and action of endothelin-1 and exerts a negative feedback on the formation of ox

LDL. Aging, injuries to the coronary endothelial layer, acceleration of endothelial turnover by risk factors [Western diet, pollution, smoking, diabetes, and hypertension] result in areas of the coronary arteries covered with regenerated endothelium. These regenerated cells differ from their native counterpart because of accelerated cell senescence, leading to a reduced production abnormal of NO. This then permits the inflammatory reaction leading to atherosclerosis (39, 40), in particular in patients with high cholesterol plasma levels.

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Legends to Figures

Figure 1 Postulated G-protein mediated signal transduction processes in a normal, native endothelial cell. Activation of the cell causes the release of nitric oxide (NO), which has important protective effects in the vascular wall. Abbreviations: 5-HT, serotonin receptor; B, bradykinin receptor; P, purinoceptor; G, coupling proteins [From Ref. 13. By permission].

Figure 2 Effects of oxidized low-density lipoproteins (OXLDL) in a regenerated endothelial cell, resulting in the reduced release of nitric oxide (NO). Abbreviations: 5-HT, serotonin receptor; B, bradykinin receptor; P, purinoceptor; G, coupling proteins [From Ref. 13. By permission].

Figure 3 Mechanisms of oxLDL-induced impairment of endothelial NO production. The NO synthase (NOS) uses L-arginine to generate NO. NO production could be attenuated in the presence of oxLDL by interfering with the supply of L-arginine to the enzyme through endogenous competitive inhibitors such as asymmetrical dimethyl-L-arginine (ADMA) as well as degradation of arginine through arginase. NOS expression and specific activity are decreased by oxLDL through RhoA and PKC. NO bioavailability is reduced by an oxLDL-mediated activation of the NADPH oxidase, which leads to superoxide anion (O_2^-) formation. This process facilitates the generation of peroxynitrite ($ONOO^-$), which subsequently oxidizes tetrahydrobiopterin (BH_4) of NOS, leading to NOS uncoupling. Uncoupled NOS itself produces O_2^-, further promoting the process of BH_4 oxidation. Rho, member of the Rho protein family (either RhoA or Rac) [From Ref.13. By permission].