Current perspectives

Coronary endothelial dysfunction after ischemia and reperfusion and its prevention by ischemic preconditioning

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In the coronary circulation, when reperfusion follows ischemia, endothelial dysfunction occurs. This is characterized by a reduced endothelial release of nitric oxide and by an increased release of reactive oxygen species and endothelin. The reduced availability of nitric oxide leads to the adhesion of neutrophils to the vascular endothelium, platelet aggregation and, with the contribution of endothelin, vasoconstriction, which are responsible for the "no-reflow" phenomenon. Neutrophil adhesion is followed by the release of the superoxide anion from neutrophils and endothelial cells. Preconditioning limits the endothelial damage by ischemia-reperfusion. A relevant role is attributed to the increased endothelial release of nitric oxide, while that of adenosine is controversial. Another effect of preconditioning on the coronary vasculature is the acceleration of vasodilation in reactive hyperemia after a brief coronary occlusion. The acceleration is prevented if myocardial protection is achieved by means of the activation of the mitochondrial adenosine triphosphate sensitive potassium channels by diazoxide and persists when ischemic preconditioning is induced after blockade of the same channels by 5-hydroxydecanoate.

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The injuries caused by ischemia and reperfusion (I/R) affect both the myocardium and coronary microvasculature[1-8]. Injuries of the coronary vasculature lead to the so called endothelial dysfunction, i.e. to an alteration of the function of the vascular endothelial cells, which has been observed after ischemia followed by reperfusion as well as in several different clinical conditions, such as arterial hypertension, preeclampsia, diabetes, atherosclerosis, heart failure, and coronary artery diseases[9-12].

In I/R not only the preservation of myocardial function, but also the prevention of endothelial dysfunction is an important therapeutic goal. Among the various approaches to preserve both the myocardial and vascular functions, ischemic preconditioning, i.e. one or more brief periods of ischemia which precede prolonged I/R, provides the background for promising interventions[7,8,13-18].

Endothelial dysfunction: general aspects

In general, endothelial dysfunction is a condition characterized by a reduced endothelial production of nitric oxide (NO) and by an increased release of reactive oxygen species (ROS) and, possibly, of endothelin[7,8,14,19,20].

Since NO not only causes vasodilation but also prevents platelet aggregation and the expression of the adhesion molecule, in the microvasculature endothelial dysfunction leads to adhesion of neutrophils to the endothelium, endothelial injury, platelet aggregation, and possible impairment of blood flow[21-24]. While in peripheral tissues leukocyte adhesion takes place mainly in the post-capillary venules, in the heart which has been exposed to I/R the adhesion of neutrophils is also observed in the coronary arterioles and capillaries[25,26].

In general, the reduced release of NO is also responsible for an impaired response of the resistance vessels to vasodilator stimuli as has been observed in experimental models[27] and in some pathological conditions[28].

ROS are mainly released during I/R in both endothelial cells and neutrophils. While in the endothelial cells the production of superoxide anion ($O_2^{-}\cdot$) is the result of the activity exerted by xanthine oxidase

on the hypoxanthine produced by the myocardium and other cells during the period of ischemia, in neutrophils it is due to the activity of the enzyme NADPH hydrogenase on molecular oxygen[29,30].

Finally, the role of endothelin has been highlighted by the fact that even the blockade of endothelin receptors has been found to limit the endothelial damage brought about by I/R[19,20].

Endothelial dysfunction after ischemia-reperfusion

The first report indicating that myocardial ischemia followed by reperfusion leads to coronary endothelial dysfunction was published in 1982[31]. In the anesthetized dog, it was demonstrated that, after 90 min of myocardial ischemia followed by 1 or 2 hours of reperfusion, the vasodilator response of the coronary vasculature to thrombin[31] was reduced. Later on it was shown that even the vasodilation induced by acetylcholine is limited by I/R[32].

In case of I/R, the impairment of the endothelial release of NO is also considered one of the pivotal steps of the pathway leading to endothelial dysfunction[5,22]. Since in the isolated rat heart it was found that the infusion of the ROS scavenger N-2-mercaptopropionyl glycine before I/R results in the prevention of the coronary endothelium-dependent relaxation by acetylcholine, the limitation of NO availability has been, at least in part, attributed to the activity of $O_2^{-}\cdot$ formed during reperfusion in the endothelial cells and neutrophils[14,22,23]. As soon as it is produced, $O_2^{-}\cdot$ acts as a scavenger of NO to which it combines to form peroxynitrite ($ONOO^-$). Both $O_2^{-}\cdot$ and $ONOO^-$ can cause marked structural injuries of the vascular endothelial cells[3,22,23,33], which show extensive swelling and disruption with a severely impaired NO release. Moreover, NO synthase itself may produce ROS (i.e. $O_2^{-}\cdot$ and H_2O_2) as occurs in case of a reduced tetrahydrobiopterin (BH_4) availability[34]. The reduction in BH_4 may occur in several pathophysiological conditions including coronary disease and I/R[35].

The reduced availability of NO persists for hours or days and is responsible for an upregulation of the cellular adhesion molecules[22,33], which causes an interaction between neutrophils and endothelial cells which in turn leads to a worsening of the endothelial injuries and to myocardial damage. Cellular adhesion molecules are classified in three groups: selectins, β_2-integrins, and immunoglobulins[36-45] (Table I).

The main steps characterizing the neutrophil-endothelial cell interaction are the rolling of neutrophils on the endothelium, their activation and adhesion to the endothelial cells and their infiltration into the underlying myocardium[22,46-48]. Neutrophil infiltration into the myocardium is revealed by a marked increase in the cardiac myeloperoxidase activity. Mast cells have been suggested to contribute to such an interaction through the release of histamine, serotonin, platelet activating factor, and tumor necrosis factor-α[49].

In the first step, neutrophils roll slowly along the vessel wall thus increasing the probability of contact with the endothelial cells[49]. Rolling is favored by P- L- and E-selectins, which are upregulated on the membrane of the endothelial cells even in response to an increased release of histamine after ischemia[22,49-53]. It is noticeable that the synergism between neutrophils and platelets, mediated by CD11b/CD18 and glycoprotein IIb/IIIa complexes (Table I) or by an interaction between sialylated oligosaccharides (Sialyl $Lewis^X$) and P-selectin, has been demonstrated to significantly contribute to the post-reperfusion cardiac dysfunction[23,54,55].

The second step consists of the activation of neutrophils and their adhesion to endothelial cells. Activation has been attributed to the upregulated expression of the β_2-integrin CD11b[56], while adhesion is attributed to the binding of the β_2-integrin CD18 to the intercellular adhesion molecule-1 (ICAM-1)[48,56].

Table I. Adhesion molecules.

Family	Location	Stimuli/expression
Selectins		
E-selectin	Endothelial cells	IL-1, TNF-α and LPS
L-selectin	Leukocytes	Constitutively exposed
P-selectin	Platelets and endothelial cells	Inflammatory agents
β_2-integrins		
CD18 (CD11a, CD11b or Mac-1, CD11c)	Leukocytes	Constitutively exposed
GP IIb/IIIa	Platelets and endothelial cells	Constitutively exposed
Immunoglobulins		
ICAM-1	Endothelial cells	IL-1 and TNF-α
VCAM-1		IL-1 and TNF-α
PECAM-1	Neutrophils, monocytes, platelets and endothelial cells	Constitutively exposed

GP = glycoprotein; ICAM-1 = intercellular adhesion molecule-1; IL-1 = interleukin-1; LPS = lipopolysaccharide; PECAM-1 = platelet endothelial cell adhesion molecule-1; TNF-α = tumor necrosis factor-α; VCAM-1 = vascular cell adhesion molecule-1.

The adhesion of neutrophils is followed by the third step of the interaction between granulocytes and the endothelium, which allows the platelet endothelial cell adhesion molecule- and vascular cell adhesion molecule-mediated infiltration of neutrophils among myocardial fibers[57-59].

The adhesion of neutrophils to the endothelial cells can cause the occlusion of the capillary bed which in turn contributes to the impairment of the coronary blood flow, i.e. of the so called "no-reflow" phenomenon[1-3]. The no-reflow phenomenon consists in a progressive reduction of flow which takes place during the reperfusion in myocardial areas which were adequately perfused immediately after the reopening of a coronary occlusion[1,21]. The reduced release of the vasodilator NO and the increased production of ROS, $ONOO^-$ and endothelins contribute to the no-reflow phenomenon. The lack of NO leads to the no-reflow phenomenon through vasoconstriction, platelet aggregation and vasospasm, and possibly through the activation of neutrophils and their adhesion to the endothelium (Fig. 1). The presence of a residual coronary stenosis and/or vasoconstriction of large epicardial vessels reduces the perfusion pressure into the capillaries where the adhesive forces exceed the pressure gradients which normally allow the blood to flow. Molecules released during I/R, such as angiotensin II, histamine and tumor necrosis factor-α which promote cytokine release, cell adhesion molecule expression and neutrophil recruitment also contribute to the occurrence of no-reflow. Among these molecules, angiotensin II may play a pivotal role as it is also a potent vasoconstrictor. Taken together all these various molecules and mechanisms lead to the severe microvascular obstruction triggered by the post-ischemic reflow[1-3,21,60].

For a given duration of occlusion, the extent of the no-reflow phenomenon has been seen to be correlated with the duration of reperfusion. Anesthetized dogs, subjected to 90 min of occlusion of the left circumflex coronary artery, showed an area of low reflow averaging 25-30% of the risk area after 2 min of reperfusion whereas it was 3 times as large after 3.5 hours of reperfusion[21]. In the same model it has also been observed that after 3.5 hours of reperfusion, the severe reduction of flow was extended to the myocardial areas which were adequately reperfused earlier after the release of

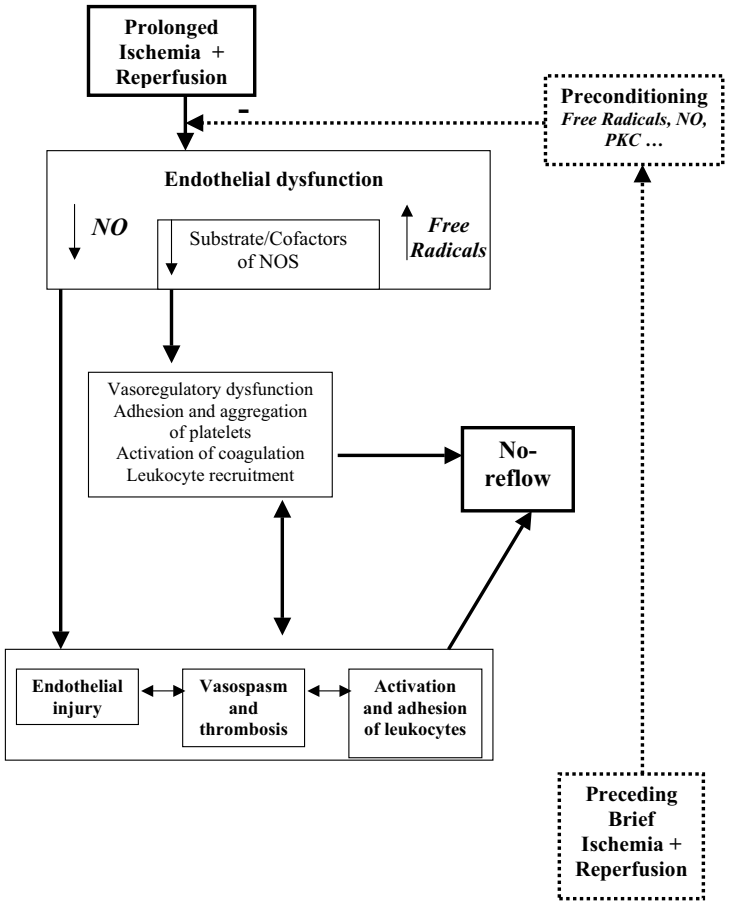

Figure 1. *The no-reflow phenomenon occurs after a prolonged period of ischemia followed by reperfusion. The possible pathway of vascular injury leading to the no-reflow phenomenon is indicated by the* thick lines *and* solid frames. *The principal steps of ischemic preconditioning correspond to the* dotted arrows *and* frames. *The* horizontal dotted arrow *indicates how ischemic preconditioning limits the endothelial dysfunction induced by a prolonged ischemia plus reperfusion. NO = nitric oxide; NOS = nitric oxide synthase; PKC = proteinkinase C.*

the occlusion. The time-course of the no-reflow phenomenon was also studied in a rabbit model[60]. In this animal species, the no-reflow area shows a rapid enlargement during the first hour after the beginning of reperfusion and continues to increase up to the eighth hour.

The three steps of neutrophil-endothelial cell interaction have also been observed in inflammatory reactions[61]. One of the first signs of vascular disease directly or indirectly related to inflammation is very often an endothelial dysfunction characterized by a reduced NO availability and a subsequent altered response to endothelium-dependent vasodilator stimuli. As a matter of fact, inflammatory markers such as the plasma levels of C-reactive protein are recognized as independent predictors of future endothelial dysfunction, myocardial dysfunction, stroke, peripheral artery disease, and cardiovascular death among individuals without any clinically evident cardiovascular disease[62]. Recent data also suggest that C-reactive protein may directly promote endothelial dysfunction by increasing the synthesis of cell adhesion molecules, by increasing monocyte chemoattractant protein-1 secretion and by facilitating macrophage low-density lipoprotein uptake[63] possibly via a direct attenuation of NO production by endothelial cells[64].

Myocardial protection by ischemic preconditioning

Ischemic preconditioning. Ischemic preconditioning is the myocardial protection which may be elicited by one or more brief occlusions of a large coronary artery. The total time of ischemia required to induce protection ranges from 2 to 20 min[65-71].

Preconditioning limits the severity of the injuries brought about by a subsequent I/R episode. Thus, after ischemic preconditioning, the extent of the area of a subsequent infarction is limited to 6-7% of the risk area[65], the incidence of I/R arrhythmias is reduced and post-ischemic myocardial stunning takes place to a lesser extent and has a shorter duration[66,72,73]. It has been seen that the effect of ischemic preconditioning consists of two phases, i.e. an initial phase or first window of protection, lasting 1-3 hours, and a late phase or second window of protection, lasting 70-90 hours. Between the two phases there is a protection-free interval that lasts 12-24 hours[13-16].

Among the various factors and mechanisms involved in the triggering of the early phase of myocardial protection, the release of adenosine and the upregulation of the endothelial NO synthase (eNOS) play a major role[13-16,71-81]. Initially it has been demonstrated that, following ischemic preconditioning, the upregulation of eNOS with the consequent increased production of NO allows protection against arrhythmias occurring during and after a prolonged I/R[71,72,81]. Later on, it was also shown that NO contributes to the preconditioning-induced limitation of the infarct size in both the early and delayed phases of protection[73-80].

Both the adenosine and NO pathways may converge to the activation/translocation of the myocardial proteinkinase C (PKC) which in turn is believed to open the adenosine triphosphate (ATP)-sensitive K^+ (K^+_{ATP}) channels. Although both the sarcolemmal and mitochondrial channels can be opened by PKC, the use of specific inhibitors and activators suggests that the protection is mediated by the mitochondrial ones via a mechanism which, to date, has not yet been fully clarified[17,82-84]. Although the PKC activation/translocation appears to be an important step in the protection induced by preconditioning ischemia and pharmacological interventions, whether the location of PKC activation is downstream and/or upstream of mitochondrial K^+_{ATP} channel opening is controversial[82-87].

Adenosine mechanism in myocardial protection. Adenosine triggers ischemic preconditioning by acting on the adenosine A_1 and A_3 membrane receptors, which in turn lead to the activation of PKC through a phospholipase-mediated release of inositol-triphosphate and diacyl-glycerol[86,88,89]. It has also been reported that in the adenosine triggered cascade to myocardial protection the generation of free radicals and the opening of the mitochondrial K^+_{ATP} channels are not required[86].

Nitric oxide as a trigger and mediator of myocardial protection. The activation of PKC can be also brought about by NO after its release has been enhanced by the upregulation of the constitutive eNOS, which has mainly been attributed to the activity of bradykinin on the endothelial B_2 receptors. Bradykinin is produced by the action of a kininogenase activated by the acidosis occurring in the vessel wall during the preconditioning ischemia[90]. It is possible that NO is also released in response to the stimulation of the vascular endothelium by adenosine[91]. The activity of NO on PKC is likely to require the production of $ONOO^-$ that is formed by the combination of NO with $O_2^{-}\cdot$. The activation of the ε isoform of PKC by $ONOO^-$ has also been suggested to be the triggering mechanism of the signaling cascade to the second window of protection. The cascade, which includes the activation of a number of kinases such as tyrosin-kinase and various mitogen-activated protein kinases, as well as of nuclear factor-kappa B, terminates with the slow activation of the inducible NO synthase (iNOS) which is in turn responsible for the delayed release of NO. In both early and delayed protection, NO appears to act as a trigger and as a mediator of preconditioning. In early protection, the brief preconditioning ischemia causes the activation of eNOS. As a mediator of the protection, NO opens the mitochondrial K^+_{ATP} channels. By combining with $O_2^{-}\cdot$ to form $ONOO^-$, NO also triggers the cascade that results in the activation of iNOS. The subsequent late release of NO

is then the mediator of the delayed protection. It has been suggested that the mechanism whereby iNOS-derived NO protects the myocardium includes the activation of guanylate-cyclase[13,78,80].

Vascular protection by ischemic preconditioning

The myocardial protection exerted by ischemic preconditioning against I/R injuries is not only the result of the increased resistance to ischemia, but is also mediated by the concomitant vascular protection that prevents the coronary endothelial dysfunction, thus limiting the no-reflow phenomenon after ischemia[8,25,26,67]. Even the occurrence of thrombosis is prevented by ischemic preconditioning[7,8,14,92,93].

A number of investigations have indicated that the protection by ischemic preconditioning results in the impaired synthesis of adhesion molecules with a reduced adhesion of neutrophils to the coronary endothelium[33,88,93]. Even the changes in the vascular responsiveness to vasoconstrictor and vasodilator stimuli after ischemic preconditioning have been considered[15,17,67-70].

Role of nitric oxide in vascular injury and protection. Richard and his coworkers[33,92] showed that ischemic preconditioning prevents the I/R-induced impairment of the coronary vasodilation by acetylcholine, which is mainly mediated by NO. Since endothelial dysfunction is characterized by neutrophil adhesion caused by the expression of adhesion molecules, the authors focused their attention on the possibility of limiting the expression of ICAM-1 by preconditioning. Using cultured rat aortic endothelial cells, they found that preconditioning prevented a prolonged anoxia-reoxygenation from increasing the expression of ICAM-1 and resulted in a reduced adhesion of neutrophils[33,92]. The suppression of the protective effect by NO synthase inhibition suggested a pivotal role of NO in vascular endothelial protection. It was then shown that ischemic preconditioning protects the vascular endothelial cells against I/R injuries and that the protection is triggered by the preconditioning-induced upregulation of eNOS, which in turn prevents I/R from reducing the endothelial NO production.

Role of reactive oxygen species in vascular protection. ROS are produced during reperfusion after both prolonged, i.e. leading to infarction, and brief preconditioning ischemia. While in the former case they contribute to neutrophil activation, i.e. to a step towards endothelial dysfunction, in the latter they initiate the signaling cascade to vascular endothelial protection[33,92]. This cascade follows the reaction of $O_2^{-}\cdot$ with NO to form $ONOO^-$ which has been reported to be responsible for the activation of PKC. Among the various PKC isomers, PKCε has been reported to initiate the cascade of events that lead to iNOS activation and, consequently, to both myocardial and vascular delayed protection[13,78-80].

It may be speculated that endothelial dysfunction takes place when ROS are released in a large quantity as occurs after a prolonged ischemia in a non-preconditioned heart. It is likely that in these conditions an excess of $O_2^{-}\cdot$ is not fully removed from NO, so that, together with $ONOO^-$, it can seriously damage the vascular endothelial cells. This large amount of ROS also exerts deleterious effects on cardiac metabolism, lipid peroxidation, stunning, arrhythmias, and myocardial necrosis. On the contrary, moderate quantities of ROS, such as those released after a brief preconditioning ischemia, may exert a regulatory action on several functions. In particular, the $O_2^{-}\cdot$ may be completely removed by NO giving rise to concentrations of $ONOO^-$ that are large enough to trigger the protective cascade but insufficient to cause oxidative injuries.

Role of adenosine in vascular protection. In contrast to the pivotal role of NO in vascular preconditioning, the role of the activation of the vascular adenosine receptor is controversial. In fact, while Maczewski and Beresewicz[94] and Merkus et al.[15] report that A_2 adenosine receptor blockade prevents vascular protection by ischemic preconditioning, Kubes et al.[88] observed that adenosine-deaminase, as well as A_1 and A_2 adenosine receptor blockade are poorly effective in preventing the protection exerted by ischemic preconditioning against neutrophil rolling and adhesion and microvascular dysfunction. In partial contrast to this finding they also found that pre-treatment with exogenous adenosine reduced the vascular damage by I/R. Thus exogenous, but not endogenous, adenosine seems to take part in vascular protection. However, since the role of PKC in vascular preconditioning[25,26,33,92], as well as its activation by ischemia-produced adenosine[89] have been confirmed, it seems surprising that only exogenous adenosine may be responsible for the prevention of endothelial injuries. It may be argued that the protective effect observed by Kubes et al.[88] with exogenous adenosine depends on the dose they used which was likely to be much larger than the amount released in their model of ischemic preconditioning. This hypothesis is consistent with the finding of Zahler et al.[95] who, in the isolated perfused guinea pig heart, observed that the stimulation of the endothelial A_1 receptors with submicromolar levels of adenosine does not prevent but rather favors neutrophil adhesion, and suggested that endothelial protection may in part be related to the use of high pharmacological doses of adenosine. It may be speculated that the protection against leukocyte adhesion observed with high concentrations of adenosine is in part mediated by the endothelial release of NO[91], which is likely not to be induced by low concentrations.

An alternative explanation of the ineffectiveness of the blockade of the adenosine receptors in preventing

vascular protection by ischemic preconditioning may reside in the fact that PKC can be activated by NO and ROS independently of the adenosine pathway[85,86].

It has also been suggested that adenosine A_2, but not A_1, receptor activation may be involved in the endothelial protection induced by ischemic preconditioning. However, in addition to exogenous adenosine, even A_1 and A_3 receptor agonists may afford endothelial protection[94,96]. Since A_1 and A_3, rather than A_2 adenosine receptors, are mainly represented on the myocardial membrane, the possibility of inducing vascular preconditioning by their activation is quite surprising. Further studies are needed to ascertain the receptor subtype(s) implicated in vascular protection. Since the A_1 and A_3 receptors are implicated in myocardial protection, further investigations are also required to understand by which mechanism these receptors take part in the protection of the vasculature. At the moment, however, experiments performed in our laboratory seem to exclude the need of myocardial protection to achieve vascular preconditioning.

Finally, the presence of adenosine receptors on the neutrophil membrane prevents these cells from interacting with the endothelium and from taking part in inflammatory responses[97,98]. The action on these receptors may also contribute to vascular protection.

Coronary responsiveness to vasodilator stimuli after ischemic preconditioning

In the anesthetized dog, Thourani et al.[99] occluded for 30 min and reperfused for 3 hours the anterior descending coronary artery. Using the left circumflex artery as a control, they found that in the previously ischemic vascular bed of the anterior descending artery, the vasodilator response to acetylcholine was impaired. If the occlusion was preceded by a preconditioning ischemia no significant difference in the response was observed between the vascular beds of the two arteries. Since sodium nitroprusside was similarly effective on the anterior descending artery independently of ischemic preconditioning, the reduced vasodilator effect of acetylcholine may be attributed to a reduced NO release from the endothelial cells of the ischemic non-preconditioned group. The authors also observed that the adherence of fluorescent labeled neutrophils to the endothelium of the epicardial arteries was inhibited after preconditioning.

In our laboratory the effect of ischemic preconditioning on the coronary response to vasodilator metabolic stimuli was studied with reactive hyperemia[69,70]. These experiments induced us to exclude a role of myocardial preconditioning as a step towards endothelial protection.

In the anesthetized goat, reactive hyperemia in the left circumflex artery was induced in controls and after ischemic preconditioning. After preconditioning, the pattern of reactive hyperemia following 15 s of occlusion showed two remarkable changes, i.e. a reduction of the total hyperemic flow and an acceleration of the vasodilation immediately after the removal of the occlusion. The latter effect being revealed by a 50% reduction in the time to the peak hyperemia in the presence of an unchanged level of the peak (Fig. 2). Then, the preliminary administration of an adenosine A_1 receptor blocking agent prevented the reduction in the total hyperemic flow, while only the administration of a NO synthase inhibitor suppressed the acceleration of the vasodilation. These findings suggested that ischemic preconditioning could be responsible for the acceleration of the vasodilation via an increased endothelial release of NO, and for the reduction in the total hyperemic flow via a decrease in myocardial metabolism brought about by the signaling cascade triggered by myocardial adenosine release. On the basis of these conclusions it was argued that ischemic preconditioning could affect reactive hyperemia by an effect (NO release) leading to vascular protection and by another effect (reduction in cardiac metabolism) related to myocardial protection. Of these two effects only the former could be considered an index of vascular preconditioning. However, since even NO release can trigger a signaling cascade leading to myocardial protection, in an attempt to investigate whether myocardial protection could contribute to the acceleration of vasodilation, experiments were performed with the administration of diazoxide and 5-hydroxydecanoate (5-HD), a specific activator and a blocker respectively of the mitochondrial K^+_{ATP} channels[17,18]. In fact, the activation of these channels is considered one of the pivotal steps to myocardial protection.

In spite of the protective effect on the myocardium, diazoxide did not produce any change in the coronary reactive hyperemia[17]. However, when induced after diazoxide, preconditioning caused the expected acceleration of vasodilation and a decrease in the total hyperemic flow which surprisingly occurred to a greater extent than in the absence of diazoxide. The results suggested that the acceleration of vasodilation, i.e. vascular preconditioning, was independent of the pathway leading to myocardial protection while the reduction in the total hyperemic flow, i.e. the coronary effect of the reduced metabolism characterizing myocardial protection, not only could be induced by an adenosine mechanism, but could also be enhanced by the preliminary activation of the mitochondrial K^+_{ATP} channels.

The absence of any role of myocardial protection in inducing or favoring vascular preconditioning was further confirmed by the ineffectiveness of 5-HD in modifying the changes induced by a brief ischemia on the coronary reactive hyperemia. Moreover, these changes appeared when the administration of 5-HD was followed by the preconditioning occlusion[18].

It has been suggested that K^+_{ATP} channels may be involved in vascular preconditioning[100] on the basis of the

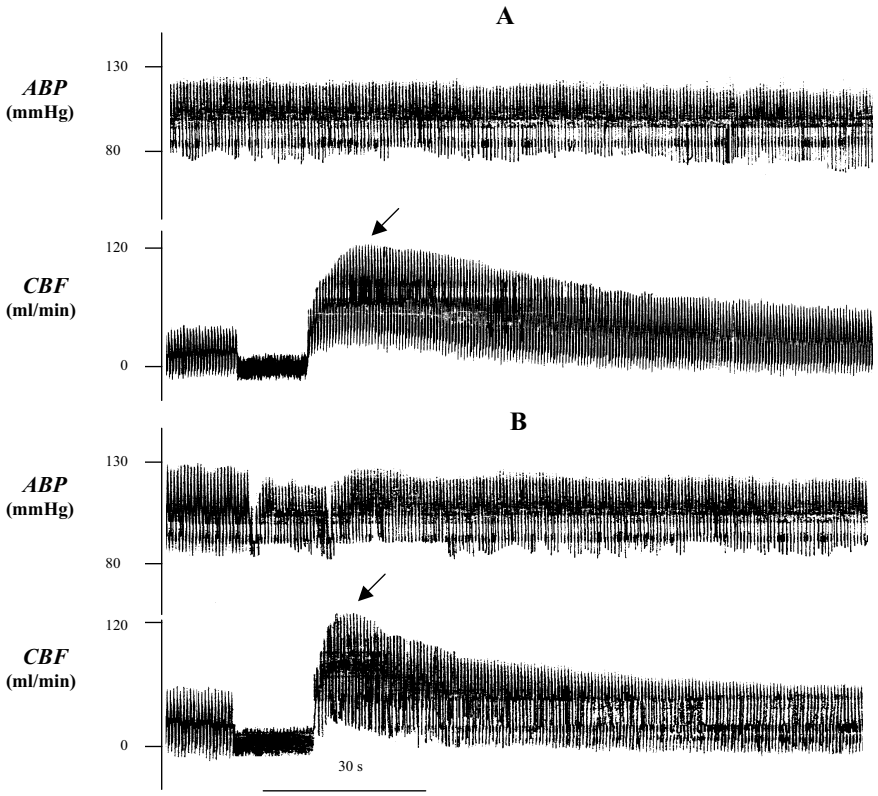

Figure 2. *The effect of ischemic preconditioning on the coronary reactive hyperemia.* Panel A: *before preconditioning.* Panel B: *after preconditioning, which was obtained with a total of 5 min of coronary occlusion. In both panels the effects of 15 s of coronary occlusion followed by reactive hyperemia are shown. The* oblique arrows *indicate the peaks of the hyperemia, which occur early after preconditioning* (panel B)*, showing an acceleration of vasodilation. After preconditioning* (panel B) *the total hyperemic flow is also reduced as may be inferred from the reduction of the area between the upper border of the coronary blood flow (CBF) and the zero flow level. ABP = aortic blood pressure. From Pagliaro et al.[18], modified.*

observation that the protection against endothelial dysfunction by ischemic preconditioning was attenuated in the presence of glibenclamide, a non-specific blocker of both mitochondrial and plasma membrane K^+_{ATP} channels. It remains to be elucidated whether this protection is achieved by the opening of the membrane channels alone or in combination with the mitochondrial K^+_{ATP} channels.

Clinical implications

Although in myocardial infarction early reperfusion has been shown to be successful in reducing mortality, reperfusion itself may increase the size of the necrotic area. In addition to the various myocardial mechanisms responsible for the reperfusion injuries, endothelial dysfunction can seriously contribute to the post-ischemic damage through the no-reflow phenomenon. Attention has thus been paid by several authors to the limitation of such a damage by the use of techniques made available by the knowledge of the mechanisms of the vascular endothelial protection elicited either by interventions during reperfusion[101-105] or by ischemic pre-conditioning[96-101,106]. In particular, the intracoronary administration of adenosine, a molecule involved in ischemic preconditioning, during reperfusion was shown to greatly improve the beneficial effect of coronary angioplasty[105]. It is also noteworthy that ischemic preconditioning has been associated with a better response to thrombolysis during acute infarction in patients with pre-infarction angina[106]. A number of investigations have been focused on the possibility of preventing endothelial dysfunction in the ischemic-reperfused heart and of limiting the neutrophil-endothelial cell interaction, platelet aggregation and vasoconstriction[54,61,93,107,108]. Monoclonal antibodies to adhesion molecules have been effective in attenuating the neutrophil-mediated injury in the mouse[61]. A chimerical Fab fragment of a monoclonal glycoprotein IIb/IIIa antibody was seen to reduce the platelet-neutrophil interaction in the guinea-pig heart, thus improving cardiac performance after I/R[54].

Diazoxide has been seen to produce myocardial protection by directly activating the mitochondrial K^+_{ATP} channels[84-87]. In our experiments it failed to induce the acceleration of vasodilation which can be taken as an index of vascular protection[17]. However, in the isolated coronary capillaries of guinea-pig hearts, diazoxide opened the K^+ channels of the endothelial cells suggesting that through this mechanism it could lead to an increase in the free cytosolic Ca^{2+} concen-

tration and activate endothelial NO synthase[109]. The fact that in our experiments no effect of an increased release of NO, i.e. no acceleration of vasodilation, was observed after diazoxide can be due to the different doses used in the intact animal[17] and in the *in vitro* experiments[109]. In fact, the doses we administered to the anesthetized goat (1.25 and 2.50 mg/kg), though sufficient to open the mitochondrial channels[17], were kept within the limits required to avoid any hypotensive effect which could result from the vascular smooth muscle relaxation brought about by the hyperpolarization (activation of the sarcolemmal K^+_{ATP} channels) of the muscle membrane and, possibly, from the release of NO from the endothelial cells. The possibility of a protective effect exerted by diazoxide on the vascular endothelium does not weaken the hypothesis that the signaling cascade leading to myocardial protection is not involved in the prevention of endothelial dysfunction. In fact, the alleged vascular protection is due to the K^+ channels located in the membrane of the smooth muscle fibers and of the endothelium, and not to those located in the mitochondrial membrane of the cardiomyocytes.

Under both normoxic and ischemic conditions, pretreatment with NO donors also mimics the protective effects of ischemic preconditioning against myocardial and vascular damage[13,16,75-80]. In particular, a protective effect by pre-treatment with nitroglycerin has recently been described in humans undergoing coronary angioplasty[77]. Following NO donor infusion, a pivotal role is played by the generation of free radicals during early and delayed protection[13,16,75-80]. In our laboratory low doses of Angeli's salt, a donor of the nitroxyl anion – the one electron reduction product of NO –, have also been seen to induce early preconditioning against myocardial damage[110]. Interestingly, the protective effects of Angeli's salt were found to be more potent than the protective effects induced by equimolar concentrations of the pure NO donor diethylamine/NO. Whether or not nitroxyl plays a role in vascular preconditioning remains to be elucidated.

The balance between ROS and NO generation is not only important in acute endothelial dysfunction, but it is also an important regulator of the cellular function in chronic conditions. For instance, in patients with coronary artery disease, the removal of ROS with ascorbic acid was effective in causing the regression of endothelial dysfunction[111]. ROS, in fact, prevent the activity of NO by combining with it to form $ONOO^-$. The protective effect of the infusion of exogenous BH_4 has also been demonstrated in conditions of an increased ROS production and of a reduced NO availability[112]. The role of ascorbic acid and of BH_4 in reversing endothelial dysfunction seems in contrast with the finding that $ONOO^-$ triggers a signaling cascade leading to the second window of myocardial protection[13,16,75-80]. This is only apparently a discrepancy because, as pointed out above, ROS and $ONOO^-$ can be beneficial when released in the triggering phase (brief preconditioning ischemia) and deleterious when released in the reperfusion phase after a prolonged ischemia, possibly because of the different amounts produced in these two phases. In fact, as said above the large amount of ROS generated during prolonged I/R exerts many deleterious effects, including the inhibition of cardiac metabolism, lipid peroxidation, stunning, arrhythmias, and possibly necrosis. On the contrary, small amounts of ROS, such as those generated during brief I/R, may not have toxic effects, but rather they may exert a regulatory action on several cell functions, including the activation of the pathway leading to ischemic preconditioning. Nevertheless, negative results came from trials in which free radical scavengers such as recombinant human superoxide dismutase or vitamin E were administered to patients with coronary artery disease or risk factors for cardiovascular events[113,114]. In addition to the dual role of ROS (beneficial vs deleterious), among the reasons why these scavengers did not show any consistent benefit in these human studies the following may be included: a) the type of ROS generated (e.g. superoxide dismutase only removes the superoxide and not the hydroxyl radical); b) the site of ROS generation (most scavengers do not enter cells and they can only remove the ROS generated in the extracellular space); and c) the rate of reaction between two ROS and/or scavengers[115]. The importance of the rate of reaction can be understood if we consider that, despite a 5 times lower concentration of NO with respect to superoxide dismutase, 50% or more of the available superoxide will react with NO to form $ONOO^-$ instead of reacting with superoxide dismutase[116].

A major role in the prevention of myocardial ischemia is attributed to exercise training[107,117,118]. In particular, in patients with coronary atherosclerosis and endothelial dysfunction, training is reported to avoid paradoxical coronary constriction and to improve coronary flow in response to acetylcholine[107,119]. In fact, the increase in shear stress and pulse pressure[120] occurring during exercise enhances eNOS expression and activity, while the increased cardiac work induces the release of adenosine from the endothelium. Both NO and adenosine lead to endothelial and myocardial protection against I/R injuries. However, the fact that exercise training improves the coronary circulation in patients with atherosclerosis clearly indicates that adequate and constant physical activity may also represent a treatment of a preexistent endothelial dysfunction which could otherwise lead to myocardial infarction.

It is noteworthy that during exercise even the production of ROS increases[107,121]. It is not clear at the moment whether in trained individuals the beneficial effect of exercise is due to the ability of ROS to induce preconditioning or to an increased production of scavengers which prevent the deleterious oxidative stress by ROS *per se,* or both.

Conclusions

A prolonged I/R can cause both myocardial injuries and coronary endothelial dysfunction, the latter being in turn responsible for a worsening of the former. Endothelial dysfunction, which consists of the neutrophil-endothelial cell interaction, platelet aggregation and vasoconstriction, i.e. of the processes which lead to the no-reflow phenomenon, can be limited by a preliminary ischemic preconditioning via an enhanced release of NO and, in part, of adenosine, which acts on the vascular A_2 receptors. In addition to this protective effect, ischemic preconditioning improves the responsiveness of the coronary vasculature to vasodilator stimuli. It is noticeable that, though triggered by the same maneuver causing myocardial preconditioning, coronary vascular endothelial preconditioning is sustained by a signaling cascade different from the one leading to myocardial protection.

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References

1. Camilleri JP, Fabiani JN. No-reflow phenomenon and acute myocardial ischemia. The need for further investigation. Biomedicine 1977; 20: 353-6.
2. Forman MB, Puett DW, Virmani R. Endothelial and myocardial injury during ischemia and reperfusion: pathogenesis and therapeutic implications. J Am Coll Cardiol 1989; 13: 450-9.
3. Forman MB, Virmani R, Puett DW. Mechanism and therapy of myocardial reperfusion injury. Circulation 1990; 81 (Suppl): IV69-IV78.
4. Pearson PJ, Schaff HV, Vanhoutte PM. Acute impairment of endothelium-dependent relaxations to aggregating platelets following reperfusion injury in canine coronary arteries. Circ Res 1990; 67: 385-93.
5. Tsao PS, Aoki N, Lefer DJ, Johnson D, Lefer AM. Time course of endothelial dysfunction and myocardial injury during myocardial ischemia and reperfusion in the cat. Circulation 1990; 82: 1402-12.
6. Tsao PS, Lefer AM. Time course and mechanism of endothelial dysfunction in isolated ischemic- and hypoxic-perfused rat hearts. Am J Physiol 1990; 259: H660-H666.
7. DeFily DV, Chilian WM. Preconditioning protects coronary arteriolar endothelium from ischemia-reperfusion injury. Am J Physiol 1993; 265: H700-H706.
8. Kaeffer N, Richard V, François A, Lallemand F, Henry JP, Thuillez C. Preconditioning prevents chronic reperfusion-induced coronary endothelial dysfunction in rats. Am J Physiol 1996; 271: H842-H849.
9. Frohlic ED. State of the Art Lecture. Risk mechanisms in hypertensive heart disease. Hypertension 1999; 34: 782-9.
10. Bell DS. Inflammation, insulin resistance, infection, diabetes and atherosclerosis. Endocr Pract 2000; 6: 272-6.
11. Page NM. The endocrinology of pre-eclampsia. Clin Endocrinol 2002: 57: 413-23.
12. Russo G, Leopold JA, Loscalzo J. Vasoactive substances: nitric oxide and endothelial dysfunction in atherosclerosis. Vascul Pharmacol 2002; 38: 259-69.
13. Bolli R. The early and late phase of preconditioning against myocardial stunning and the essential role of oxyradicals in the late phase: an overview. Basic Res Cardiol 1996; 91: 57-63.
14. Kaeffer N, Richard V, Thuillez C. Delayed coronary endothelial protection 24 hours after preconditioning. Role of free radicals. Circulation 1997; 96: 2311-6.
15. Merkus D, Stepp DW, Jones DW, Nishikawa J, Chilian WM. Adenosine preconditions against endothelin-induced constriction of coronary arterioles. Am J Physiol 2000; 279: H2593-2597.
16. Pagliaro P, Gattullo D, Rastaldo R, Losano G. Ischemic preconditioning: from the first to the second window of protection. Life Sci 2001; 69: 1-15.
17. Pagliaro P, Rastaldo R, Losano G, Gattullo D. Mitochondrial ATP-sensitive channel opener does not induce vascular preconditioning, but potentiates the effect of a preconditioning ischemia on coronary reactive hyperemia in the anesthetized goat. Pflugers Arch 2001; 443: 166-74.
18. Pagliaro P, Chiribiri A, Rastaldo R, et al. Ischemic preconditioning changes the pattern of coronary reactive hyperemia regardless of mitochondrial ATP-sensitive K^+ channel blockade. Life Sci 2002; 71: 2299-309.
19. Halcox JP, Nour KR, Zalos G, Quyyumi AA. Coronary vasodilation and improvement in endothelial dysfunction with endothelin ET_A receptor blockade. Circ Res 2001; 89: 969-76.
20. d'Uscio LV, Barton M, Shaw S, Luscher TF. Chronic ET_A receptor blockade prevents endothelial dysfunction of small arteries in apolipoprotein E-deficient mice. Cardiovasc Res 2002; 53: 487-95.
21. Ambrosio G, Weisman HF, Mannini JA, Becker LC. Progressive impairment of regional myocardial perfusion after initial restoration of postischemic blood flow. Circulation 1989; 80: 1846-61.
22. Lefer AM, Lefer DJ. The role of nitric oxide and cell adhesion molecules on the microcirculation in ischemia-reperfusion. Cardiovasc Res 1996; 32: 723-51.
23. Lefer AM, Campbell B, Scalia R, Lefer DJ. Synergism between platelets and neutrophils in provoking cardiac dysfunction after ischemia and reperfusion. Circulation 1998; 98: 1322-8.
24. Ambrosio G, Tritto I. Interaction between the endothelium and blood cells in acute coronary syndromes. Ital Heart J 2001; 2 (Suppl 3): 43S-44S.
25. Laude K, Thuillez C, Richard V. Coronary endothelial dysfunction after ischemia and reperfusion: a new therapeutic target? Braz J Med Biol Res 2001; 34: 1-7.
26. Laude K, Beauchamp P, Thuillez C, Richard V. Endothelial protective effects of preconditioning. Cardiovasc Res 2002; 55: 466-73.
27. Gattullo D, Pagliaro P, Linden RJ, Merletti A, Losano G. The role of nitric oxide in the initiation and in the duration of some vasodilator responses in the coronary circulation. Pflugers Arch 1995; 430: 96-104.
28. Khalil RA, Granger JP. Vascular mechanism of increased arterial pressure in preeclampsia: lessons from animal models. Am J Physiol 2002; 283: R29-R45.
29. Terada LS, Guidot M, Leff JA, et al. Hypoxia injuries endothelial cells by increasing endogenous xanthine oxidase activity. Proc Natl Acad Sci USA 1992; 89: 3362-6.
30. Houston M, Estevez A, Chumley P, et al. Binding of xanthine oxidase to vascular endothelium - kinetic characteri-

zation and oxidative impairment of nitric oxide-dependent signaling. J Biol Chem 1999; 274: 4985-94.
31. Ku DD. Coronary vascular reactivity after acute myocardial ischemia. Science 1982; 218: 576-8.
32. VanBenthuysen KM, McMurtry IF, Horwitz LD. Reperfusion after acute coronary occlusion in dogs impairs endothelium-dependent relaxation to acetylcholine and augments contractile reactivity in vitro. J Clin Invest 1987; 79: 265-74.
33. Beauchamp P, Richard V, Tamion F, et al. Protective effects of preconditioning in cultured rat endothelial cells. Effects of neutrophil adhesion and expression of ICAM-1 after anoxia and oxygenation. Circulation 1999; 100: 542-6.
34. Cosentino F, Katusic ZS. Tetrahydrobiopterin and dysfunction of endothelial nitric oxide synthase in coronary arteries. Circulation 1995; 91: 139-44.
35. Tiefenbacher CP, Chilian WM, Mitchell M, DeFily DV. Restoration of endothelium-dependent vasodilation after reperfusion injury by tetrahydrobiopterin. Circulation 1996; 94: 1423-9.
36. Geng JG, Bevilacqua MP, Moore KL, et al. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. Nature 1990; 343: 757-60.
37. Xia YF, Liu LP, Zhong CP, Geng JG. NF-kappaB activation for constitutive expression of VCAM-1 and ICAM-1 on B lymphocytes and plasma cells. Biochem Biophys Res Commun 2001; 289: 851-6.
38. Asa D, Raycroft L, Ma L, et al. The P-selectin glycoprotein ligand functions as a common human leukocyte ligand for P- and E-selectins. J Biol Chem 1995; 270: 11662-70.
39. Bevilacqua MP, Nelson RM, Mannori G, Cecconi O. Endothelial-leukocyte adhesion molecules in human disease. Annu Rev Med 1994; 45: 361-78.
40. Bevilacqua MP, Nelson RM. Selectins. J Clin Invest 1993; 91: 379-87.
41. Clemetson KJ, Clemetson JM. Integrins and cardiovascular disease. Cell Mol Life Sci 1998; 54: 502-13.
42. Gonzi G, Ardissino D. New strategies in the treatment of acute myocardial infarction. Ital Heart J Suppl 2001; 2: 1055-60.
43. Dustin ML, Springer TA. Role of lymphocyte adhesion receptors in transient interactions and cell locomotion. Ann Rev Immunol 1991; 9: 27-66.
44. DeLisser HM, Newman PJ, Albelda SM. Molecular and functional aspects of PECAM-1/CD31. Immunol Today 1994; 15: 490-5.
45. Cao G, O'Brien CD, Zhou Z, et al. Involvement of human PECAM-1 in angiogenesis and in vitro endothelial cell migration. Am J Physiol 2002; 282: C1181-C1190.
46. Butcher EC. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell 1991; 67: 1033-6.
47. Ley K, Gaehtgens P. Endothelial, not hemodynamic, differences are responsible for preferential leukocyte rolling in rat mesenteric venules. Circ Res 1991; 69: 1034-41.
48. Radi ZA, Kehrli ME Jr, Ackermann MR. Cell adhesion molecules, leukocyte trafficking, and strategies to reduce leukocyte infiltration. J Vet Intern Med 2001; 15: 516-29.
49. Kubes P, Granger DN. Leukocyte-endothelial cell interactions evoked by mast cells. Cardiovasc Res 1996; 32: 699-708.
50. Davenpeck KL, Gauthier TW, Albertine KH, Lefer AM. Role of P-selectin in microvascular leukocyte-endothelial interaction in splanchnic ischemia-reperfusion. Am J Physiol 1994; 267: H622-H630.
51. Davenpeck KL, Gauthier TW, Lefer AM. Inhibition of endothelial-derived nitric oxide promotes P-selectin expression and actions in the rat microcirculation. Gastroenterology 1994; 107: 1050-8.
52. Weyrich AS, Buerke M, Albertine KH, Lefer AM. Time course of coronary vascular endothelial adhesion molecule expression during reperfusion of the ischemic feline myocardium. J Leukoc Biol 1995; 57: 45-55.
53. Weyrich AS, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA. Monocyte tethering by P-selectin regulates monocyte chemotactic protein-1 and tumor necrosis factor-alpha secretion. Signal integration and NF-kappa B translocation. J Clin Invest 1995; 95: 2297-303.
54. Habazettl H, Hanusch P, Kupatt C. Effects of endothelium/leukocytes/platelet interaction on myocardial ischemia-reperfusion injury. Z Kardiol 2000; 89 (Suppl 9): 92-5.
55. Ott I, Neumann FJ, Gawaz M, Schmitt M, Schomig A. Increased neutrophil-platelet adhesion in patients with unstable angina. Circulation 1996; 94: 1239-46.
56. Ilton MK, Langton PE, Taylor ML, et al. Differential expression of neutrophil adhesion molecules during coronary artery surgery with cardiopulmonary bypass. J Thorac Cardiovasc Surg 1999; 118: 930-7.
57. Kurose I, Granger DN. Evidence implicating xanthine oxidase and neutrophils in reperfusion-induced microvascular dysfunction. Ann NY Acad Sci 1994; 723: 158-79.
58. Muller WA. The role of PECAM-1 (CD31) in leukocyte emigration: studies in vitro and in vivo. J Leukoc Biol 1995; 57: 523-8.
59. Schenkel AR, Mamdouh Z, Chen X, Liebman RM, Muller WA. CD99 plays a major role in the migration of monocytes through endothelial junctions. Nat Immunol 2002; 3: 143-50.
60. Reffelmann T, Kloner RA. Microvascular reperfusion injury: rapid expansion of no reflow during reperfusion in the rabbit. Am J Physiol 2002; 283: H1099-H1107.
61. Bogen S, Pak J, Garifallou M, Deng X, Muller WA. Monoclonal antibody to murine PECAM-1 (CD31) blocks acute inflammation in vivo. J Exp Med 1994; 179: 1059-64.
62. Ridker PM. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation 2001; 103: 1813-8.
63. Pasceri V, Cheng JS, Willerson JT, Yeh ET, Chang J. Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs. Circulation 2001; 103: 2531-4.
64. Verma S, Wang CH, Li SH, et al. A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. Circulation 2002; 106: 913-9.
65. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986; 74: 1124-36.
66. Pasceri V, Lanza GA, Patti G, Pedrotti P, Crea F, Maseri A. Preconditioning by transient myocardial ischemia confers protection against ischemia-induced ventricular arrhythmias in variant angina. Circulation 1996; 94: 1850-6.
67. Kim SJ, Ghaleh B, Kudej RK, Huang CH, Hintze TH, Vatner SF. Delayed enhanced nitric oxide-mediated coronary vasodilation following brief ischemia and prolonged reperfusion in conscious dogs. Circ Res 1997; 81: 53-9.
68. Deplanque D, Bastide M, Burdet R. Ischemic preconditioning of the endothelium and smooth muscle of cerebral arteries. Trends Pharmacol Sci 2000; 21: 332-3.
69. Gattullo D, Linden RJ, Losano G, Pagliaro P, Westerhof N. Ischemic preconditioning changes the pattern of coronary reactive hyperaemia in the goat: role of adenosine and nitric oxide. Cardiovasc Res 1999; 42: 57-64.
70. Pagliaro P, Penna C, Gattullo D. The effects of ischemic preconditioning on resting coronary flow and reactive hyperemia: involvement of A_1 adenosine receptors. Life Sci 1999; 64: 1071-8.

71. Lawson CS, Downey JM. Preconditioning: state of the art myocardial protection. Cardiovasc Res 1993; 27: 542-50.
72. Vegh A, Szekeres L, Parrat JR. Transient ischemic induced by rapid pacing results in myocardial preconditioning. Cardiovasc Res 1991; 25: 1051-3.
73. Vegh A, Papp JG, Parratt JR. The antiarrhythmic effects of bradykinin in anaesthetized dogs are prevented by an inhibitor of L-arginine-nitric oxide pathway. Br J Pharmacol 1993; 110: 18-9.
74. Williams MW, Taft CS, Ramnauth S, Zhao ZQ, Vinte-Johansen J. Endogenous nitric oxide (NO) protects against ischemia-reperfusion injury in the rabbit. Cardiovasc Res 1995; 30: 79-86.
75. Nakano A, Liu GS, Heusch G, Downey JM, Cohen MV. Exogenous nitric oxide can trigger a preconditioned state through a free radical mechanism, but endogenous nitric oxide is not a trigger of classical ischemic preconditioning. J Mol Cell Cardiol 2000; 32: 1159-67.
76. Xuan YT, Tang XL, Qiu Y, et al. Biphasic response of cardiac NO synthase isoforms to ischemic preconditioning in conscious rabbits. Am J Physiol 2000; 279: H2360-H2371.
77. Leesar MA, Stoddard MF, Dawn B, Jasti VG, Masden R, Bolli R. Delayed preconditioning-mimetic action of nitroglycerin in patients undergoing coronary angioplasty. Circulation 2001; 103: 2935-41.
78. Bolli R. Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research. J Mol Cell Cardiol 2001; 33: 1897-918.
79. Pagliaro P, Gattullo D, Rastaldo R, Losano G. Involvement of nitric oxide in ischemic preconditioning. Ital Heart J 2001; 2: 660-8.
80. Dawn B, Bolli R. Role of nitric oxide in myocardial preconditioning. Ann NY Acad Sci 2002; 962: 18-41.
81. Vegh A, Szekeres L, Parratt JR. Preconditioning of ischemic myocardium: involvement of L-arginine-nitric oxide pathway. Br J Pharmacol 1992; 107: 648-52.
82. Ockaili R, Emani VR, Okubo S, Brown M, Krottapalli K, Kukreja RC. Opening of mitochondrial K_{ATP} channel induces early and delayed cardioprotective effect: role of nitric oxide. Am J Physiol 1999; 277: H2425-H2434.
83. Forbes RA, Steenbergen C, Murphy E. Diazoxide-induced cardioprotection requires signaling through a redox-sensitive mechanism. Circ Res 2001; 88: 802-9.
84. Carroll R, Gant VA, Yellon DM. Mitochondrial K_{ATP} channels protects a human atrial-derived cell line by a mechanism involving free radical generation. Cardiovasc Res 2001; 51: 691-700.
85. Pain T, Yang XM, Critz SD, et al. Opening of mitochondrial K_{ATP} channels triggers the preconditioned state by generating free radicals. Circ Res 2000; 87: 460-6.
86. Cohen MV, Yang XM, Liu GS, Heusch G, Downey JM. Acetylcholine, bradykinin, opioids, and phenylephrine, but not adenosine, trigger preconditioning by generating free radicals and opening mitochondrial K_{ATP} channels. Circ Res 2001; 89: 273-8.
87. Patel HH, Gross GJ. Diazoxide induced cardioprotection: what come first, K_{ATP} channels or reactive oxygen species? Cardiovasc Res 2001; 51: 633-6.
88. Kubes P, Payne D, Ostrowsky L. Preconditioning and adenosine in I/R-induced leukocyte-endothelial cell interactions. Am J Physiol 1998; 274: H1230-H1238.
89. Liu GS, Thornton J, Van Winkle DM, Stanley AH, Olson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. Circulation 1991; 84: 350-6.
90. Parratt J, Vegh A. Pronounced antiarrhythmic effects of ischemic preconditioning. Cardioscience 1994; 5: 9-18.
91. Chilian WM, Kuo L, DeFily DV, Jones CJ, Davis MJ. Endothelial regulation of coronary microvascular tone under physiological and pathophysiological conditions. Eur Heart J 1993; 14 (Suppl I): 55-9.
92. Richard V, Kaeffer N, Tron C, Thuillez C. Ischemic preconditioning protects against coronary endothelial dysfunction induced by ischemia and reperfusion. Circulation 1994; 89: 1254-61.
93. Kurzelewski M, Czarnowska E, Beresewicz A. Endothelin in the mechanism of endothelial injury and neutrophil adhesion in the postischemic guinea-pig heart. Eur J Pharmacol 2002; 434: 95-107.
94. Maczewski M, Beresewicz A. The role of adenosine and ATP-sensitive potassium channels in the protection afforded by ischemic preconditioning against the post-ischemic endothelial dysfunction in guinea-pig hearts. J Mol Cell Cardiol 1998; 30: 1735-47.
95. Zahler S, Becker BF, Raschke P, Gerlach E. Stimulation of endothelial A_1 receptors enhances adhesion of neutrophils in the intact guinea pig coronary system. Cardiovasc Res 1994; 28: 1366-72.
96. Giannella E, Mochmann HC, Levi R. Ischemic preconditioning prevents the impairment of hypoxic coronary vasodilatation caused by ischemia/reperfusion: role of adenosine A_1/A_3 and bradykinin B_2 receptor activation. Circ Res 1997; 81: 415-22.
97. Bouma MG, Jeunhomme TM, Boyle DL, et al. Adenosine inhibits neutrophil degranulation in activated human whole blood: involvement of adenosine A_2 and A_3 receptors. J Immunol 1997; 158: 5400-8.
98. Wakai A, Wang JH, Winter DC, Street JT, O'Sullivan RG, Redmond HP. Adenosine inhibits neutrophil vascular endothelial growth factor release and transendothelial migration via A_2B receptor activation. Shock 2001; 15: 297-301.
99. Thourani VH, Nakamura M, Duarte IG, et al. Ischemic preconditioning attenuates postischemic coronary artery endothelial dysfunction in a model of minimal invasive direct coronary bypass grafting. J Thorac Cardiovasc Surg 1999; 117: 383-9.
100. Bouchard JF, Lamontagne D. Mechanisms of protection afforded by preconditioning to endothelial function against ischemic injury. Am J Physiol 1996; 271: H1801-H1806.
101. Iwakura K, Ito H, Takiuchi S, et al. Alternation in the coronary blood flow velocity pattern in patients with no-reflow and reperfused acute myocardial infarction. Circulation 1996; 94: 1269-75.
102. Neumann FJ, Blasini R, Schmitt C, et al. Effect of glycoprotein IIb/IIIa receptor blockade on recovery of coronary flow and left ventricular function after the placement of coronary-artery stents in acute myocardial infarction. Circulation 1998; 98: 2695-2701.
103. Ito H, Taniyama Y, Iwakura K, et al. Intravenous nicorandil can preserve microvascular integrity and myocardial viability in patients with reperfused anterior wall myocardial infarction. J Am Coll Cardiol 1999; 33: 654-60.
104. Iwakura K, Ito H, Nishikawa N, et al. Early temporal changes in coronary flow velocity patterns in patients with acute myocardial infarction demonstrating the "no-reflow" phenomenon. Am J Cardiol 1999; 84: 415-9.
105. Marzilli M, Orsini E, Marraccini P, Testa R. Beneficial effects of intracoronary adenosine as an adjunct to primary angioplasty in acute myocardial infarction. Circulation 2000; 101: 2154-9.
106. Andreotti F, Pasceri V, Hackett DR, Davies GJ, Haider AW, Maseri A. Preinfarction angina as a predictor of more rapid coronary thrombolysis in patients with acute myocardial infarction. N Engl J Med 1996; 334: 7-12.
107. Gielen S, Schuler G, Hambrecht R. Exercise training in

coronary artery disease and coronary vasomotion. Circulation 2001; 103: E1-E6.
108. Scalia R, Goszen ME, Jones SP, et al. Simvastatin exerts both anti-inflammatory and cardioprotective effects in apolipoprotein E-deficient mice. Circulation 2001; 103: 2598-63.
109. Langheinrich U, Mederos y Schnitzler M, Daut J. Ca^{2+}-transients induced by K^+ channel openers in isolated coronary capillaries. Pflugers Arch 1998; 435: 435-8.
110. Pagliaro P, Mancardi D, Rastaldo R, et al. Nitroxyl affords thiol-sensitive myocardial protective effects akin to early preconditioning. Free Radic Biol Med 2003; 34: 33-43.
111. Gokce N, Keaney JF, Frei B, et al. Long-term ascorbic acid administration reverses endothelial vasomotor dysfunction in patients with coronary artery disease. Circulation 1999; 99: 3234-40.
112. Cosentino F, Patton S, d'Uscio LV, et al. Tetrahydrobiopterin alters superoxide and nitric oxide release in prehypertensive rats. J Clin Invest 1998; 101: 1530-7.
113. Flherty JT, Pitt B, Gruber JW, et al. Recombinant human superoxide dismutase (h-SOD) fails to improve recovery of ventricular function in patients undergoing coronary angioplasty for acute myocardial infarction. Circulation 1999; 89: 1982-91.
114. The Heart Outcomes Prevention Evaluation (HOPE) Study Investigators. Vitamin E supplementation and cardiovascular events in high risk patients. N Engl J Med 2000; 342: 154-60.
115. Kloner RA, Jennings RB. Consequences of brief ischemia: stunning, preconditioning and their clinical implications. Circulation 2001; 104: 2981-9.
116. Estevez AG, Jordan J. Nitric oxide and superoxide, a deadly cocktail. Ann NY Acad Sci 2002; 962: 207-11.
117. Ehsani AA, Heath GW, Hagberg JM, et al. Effects of 12 months of intensive exercise training on ischemic ST-segment depression in patients with coronary artery disease. Circulation 1981; 64: 1116-24.
118. Schuler G, Hambrecht R, Schlierf G, et al. Regular physical exercise and low-fat diet. Effects on progression of coronary artery disease. Circulation 1992; 86: 1-11.
119. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986; 315: 1046-51.
120. Pagliaro P, Senzaki H, Paolocci N, et al. Specificity of synergistic coronary flow enhancement by adenosine and pulsatile perfusion in the dog. J Physiol 1999; 520: 271-80.
121. Fukai T, Siegfried MR, Ushio-Fukai M, Cheng Y, Kojda G, Harrison DG. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. J Clin Invest 2000; 105: 1631-9.