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Endothelial Dysfunction and Vascular Preconditioning

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The prolonged occlusion of a coronary artery followed by reperfusion causes deleterious effects known as "ischemia-reperfusion injuries". In fact, these injuries, which affect both myocardium and coronary endothelium, initiate during ischemia and worsen during reperfusion. Thus, although myocardial infarction is the necrosis occurring as a consequence of the ischemia, its size can increase with reperfusion. Usually two hours of reperfusion are sufficient to induce a noticeable increase in size of the infarct area. In addition to necrosis, also myocardial stunning and arrhythmias are features of the damage.

The *endothelial dysfunction (ED)* is a structural and functional alteration of vascular endothelium that occurs during the reperfusion of the ischemic tissue. Following ischemia and reperfusion (I/R), superoxide anion (O_2^-) released during reperfusion by neutrophils and endothelial cells, removes nitric oxide (NO) to which it combines to form peroxynitrite ($ONOO^-$). Both O_2^- and $ONOO^-$ cause structural injuries to the vascular endothelial cells, which shows swelling and disruption with severe impairment of NO release. (Kaeffer et al., 1997; Beauchamp et al., 1999). Thus, vascular dysfunction by reperfusion is characterized by a reduction of NO availability due to both a decreased endothelial release and the scavenger activity of superoxide anion produced during reperfusion. Following ischemia and reperfusion (I/R), the impaired NO production is responsible for the *up-regulation* of cell adhesion molecules which induce the adhesion of leucocytes to the endothelium and allow their migration into the myocardial tissue. These molecules are *b-integrins, selectins* and *immunoglobulins*. Immunoglobulins include *intercellular adhesion molecules-1* (ICAM-1), *vascular cell adhesion molecules* (VCAM) and *platelel-endothelial cell adhesion molecules-1* (PECAM-1). The administration of exogenous NO before I/R prevents ED, thus confirming the importance of nitric oxide deficiency in the alterations induced by I/R in the vascular endothelial cells.

Ischemic preconditioning (IP) was initially seen to limit the myocardial damage caused by I/R (Murry et al. 1986). Although the classical preconditioning is obtained with one or more brief episodes of coronary occlusion for a total duration ranging from 2.5 to 20 min, the same protection is also achieved with other manoeuvres causing myocardial hypoxia, as well as by treatment with drugs which activate the signalling cascade that links hypoxia to the opening of mitochondrial ATP-sensitive potassium channels. In fact, the opening of these channels is considered to play a pivotal role in mediating myocardial preconditioning. Hypoxia triggers the signalling cascade by inducing the myocardial release of adenosine and the endothelial synthesis of nitric oxide. Since the cascade is characterised by an activation of *protein-kinase C* (PKC), also the activation of this enzyme by phorbol esters mimics IP. Myocardial protection has a peculiar time-course, as an initial phase, lasting 1-3 hours is followed by a period without protection lasting 20-24 hours before a delayed protection occurs again for and persists for 70-90 hours. Ischemic preconditioning also limits coronary endothelial dysfunction by the reduction of leucocyte adhesion to the endothelial cells and infiltration through the vessel wall. This effect is one of the aspects of the so-called *vascular preconditioning*. Since in I/R the activation of the adhesion molecules depends on the reduced availability of NO occurring during reperfusion, vascular protection can be triggered by the up-regulation of endothelial NO-synthase (NOS) with an increase of NO release, as underlined by the observation that not only the myocardial but also the vascular protective effect of IP is abolished by NOS inhibitors.

If I/R is preceded by preconditioning NO is present in such an amount to act as a scavenger of O_2^- to form $ONOO^-$, which can activate the isoform e of PKC (PKC-e), which in turn triggers a slow cascade resulting in the late activation of an inducible NOS responsible for the delayed myocardial and vascular protection.

Chronic endothelial dysfunction does not need ischemia and reperfusion. Usually it is initiated by the oxidation of low density lipoproteins (LDL) to oxidised LDL (Ox-LDL) and leads to a chronically decreased availability of NO. The consequent long term activation of adhesion molecules alters the vascular wall thus inducing the production of atherosclerotic plaques. By inhibiting the expression of the adhesion molecules an enhanced production of NO represents a valuable mechanism of protection against chronic endothelial dysfunction. The type of preconditioning which causes a long term increase of NO production is represented by constant and repeated physical exercise.

The importance of NO in coronary protection is consistent with the acceleration of vasodilatation that characterises a coronary reactive hyperemia induced after IP, as observed in the anesthetised goat (Gattullo et al. 1999). These acceleration can be prevented by NOS inhibitors but not by adenosine receptor blockers. It cannot be elicited by direct activation of mitochondrial ATP-sensitive potassium channels

by *diazoxide* and is still present after the blockade of the same channels by *5-hydroxydecanoate*, thus indicating that, though both initiated by the same brief coronary occlusion, vascular preconditiong is independent of myocardial protection (Pagliaro et al. 2002). Although this effect has no protective importance, it confirms the role of nitric oxide in vascular preconditioning.

References

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