THE ROLE OF L-ARGININE / NITRIC OXIDE PATHWAY IN ATHEROSCLEROSIS AND ISCHEMIC HEART DISEASE

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Gazi Medical Journal 1998; 9 (Suppl 1) : S14-S19

Discoveries in the past decade have revealed that the vascular endothelium plays an important role in maintaining cardiovascular function in health and disease. One of the many important functions of the endothelium is the regulation of vascular tone by the production of various biologically active substances. Furchgott and Zawadzki (1) demonstrated that the vascular relaxation induced by acetylcholine (ACh) was dependent on the presence of the endothelium which indicated the existence of an endothelium-derived relaxing factor (EDRF). Bioassay studies demonstrates that EDRF is a very labile, short-lived nonprostanoid substance (2, 3) and exerts characteristics similar to nitric oxide (NO) (4).

NO is synthesized from the terminal guanido nitrogen of the amino acid L-arginine by the enzyme NO synthase (NOS) (4). NOS is a family of enzymes with three major isoforms, neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). nNOS and eNOS are calcium/calmodulin-dependent and are usually constitutively present in cerebellar neurons and in endothelial cells, respectively (4, 5). iNOS, on the other hand, is independent of ionic calcium and its expression is induced in various types of cells, including vascular smooth muscle cells and myocytes, after stimulation with e.g. cytokines or lipopolysaccharides (6-8). The NO produced by eNOS acts intracellularly by increasing levels of cyclic guanosine monophosphate (cGMP) which results in vasorelaxation. Other important actions of NO are inhibition of adhesion and activation of neutrophils and platelets as well as inactivation of oxygen free radicals (4, 5). iNOS, on the other hand, produces high levels of NO which can be cytotoxic and is involved in inflammatory reactions (9).

Based on the biological actions of NO, impaired production and release of NO may thus be an important factor in the development and progression of cardiovascular diseases. In this paper, recent findings regarding the alterations in the L-arginine/NO pathway in cardiovascular diseases with special emphasis on atherosclerosis and myocardial ischemia are reviewed.

Atherosclerosis

Endothelium-dependent arterial relaxations are impaired in animals with experimentally induced atherosclerosis (10, 11). This effect has been demonstrated to be due to reduced release of NO. On the other hand, endothelium-independent relaxations induced by e.g. nitroglycerine are not affected in atherosclerosis indicating selective impairment of endothelium-dependent relaxation (12). The clinical consequences of these experimental findings have been elucidated in patients with coronary artery disease by intracoronary administration of the endothelium-dependent vasodilator ACh. It was demonstrated that the coronary dilator effect of ACh in normal epicardial arteries was absent or converted to constriction in atherosclerotic arteries (13, 14). Interestingly, the endothelial dysfunction does not seem to require manifest atherosclerosis but is also evident in angiographically normal vessels of patients with evidence of atherosclerosis elsewhere in the coronary arteries. Furthermore, endothelial dysfunction is also found in patients with risk factors for atherosclerosis like

hypercholesterolemia (15, 16). These findings indicate that a pathological response to ACh demonstrates early evidence of endothelial dysfunction before angiographically detectable atherosclerosis has developed.

The biological significance consequences of impaired NO release in atherosclerosis include increased adhesion of platelets which may lead to thrombus formation and release of vasospastic substances from the platelets. Lack of NO will also increase adhesion and migration of leukocytes which in turn will generate oxygen-derived free radicals and increased levels of oxidized LDL. Accumulation of oxidized LDL will further impair NO release (17).

The mechanism underlying the impaired endothelial function in atherosclerosis is still unclear. Some results suggest that the production of NO is reduced. Thus, enhanced levels of endogenous NOS inhibitors like dimethyl-L-arginine (18) are increased by cholesterol (19). Moreover, oxidized LDL may decrease the expression of eNOS (20). Other observations indicate that NO in atherosclerotic arteries is enhanced (17). This would suggest that the endothelial dysfunction is related to enhanced inactivation of NO by oxygen-derived free radicals and oxidized LDL.

Administration of the precursor for NO formation, L-arginine, in order to increase the endogenous production of NO has been tested in both experimental animals and in patients. Acute administration of L-arginine to animals and humans with hypercholesterolemia and atherosclerosis have resulted in improved endothelial function. Thus, in patients with hypercholesterolemia, intracoronary administration of L-arginine restored endothelium-dependent dilatation to ACh (15). Similar results were obtained in patients with microvascular angina and normal arteriograms (21). Furthermore, oral supplementation with L-arginine was associated with a significant improvement of endothelium-dependent vasodilatation in hypercholesterolemic rabbits (22). It was also demonstrated that oral L-arginine prevented intimal thickening and macrophage infiltration in coronary arteries (23).

Myocardial ischemia and reperfusion

The early period of reperfusion following myocardial ischemia has been demonstrated to be characterized by endothelial damage and impairment of NO release from the coronary endothelium (24, 25). Endothelial dysfunction occurs during the first minutes of reperfusion and is completed before myocardial necrosis has developed. The loss of NO release may promote adhesion of leukocytes to the endothelium, infiltration of leukocytes into the myocardium with subsequent release of myeloperoxidase and formation of oxygen-derived free radicals (Fig. 1). These features all characterize the pathology of the ischemia/reperfusion injury leading to myocardial necrosis. Attempts have therefore been made to protect the myocardium during ischemia/reperfusion by increasing the levels of NO. Administration of L-arginine, to enhance endogenous NO production, has been demonstrated to reduce the extent of myocardial ischemic and reperfusion injury under in vitro as well as in vivo conditions. Thus, under in vivo conditions L-arginine reduce the final infarct size produced by coronary artery ligation followed by reperfusion (26, 27). The effect of L-arginine is mediated via a local action in the ischemic/reperfused myocardium (26). In isolated buffer-perfused rat hearts, L-arginine given before ischemia improves myocardial recovery and coronary flow and preserves endothelial function (28). Co-administration of the NOS inhibitor nitro-L-arginine (L-NNA) abolished the cardioprotective effect of L-arginine (26, 28) indicating that the protective effect was related to NO formation. Consistent with these findings, administration of NO donors (28, 29) and authentic NO (30) were proven cardioprotective during experimental ischemia and reperfusion. These results collectively indicate that maintenance of NO release is an important factor protecting from reperfusion injury (Fig. 1).

Other studies have suggested that NO is cardiotoxic, based on observations that NOS inhibitors reduce infarct size and improve functional recovery following ischemia/reperfusion in the rabbit (31-33). It was thus suggested that formation of NO during ischemia/reperfusion may have detrimental effect on the myocardium. These opposing results may be due to differences in the rate of NO production and the type of NOS that is active under different experimental conditions. Based on the nature of different NOS isoforms, Ca2+-dependent NOS and Ca2+-independent iNOS, it is possible that

maintenance of basal production of NO by Ca2+-dependent NOS, on one hand, is protective, whereas overproduction of NO by activation of iNOS, on the other hand, is cytotoxic and detrimental to myocardial function and recovery. Therefore, studies have been performed in order to correlate the NOS activity in the heart with the functional recovery during ischemia/reperfusion. A significant Ca2+-dependent enzyme activity, suggesting eNOS, was detected in hearts undergoing non-ischemic perfusion. This activity was lost during myocardial ischemia/reperfusion, indicating that myocardial ischemia/reperfusion reduced the activity of Ca2+-dependent NOS which resulted in reduced production and release of NO during reperfusion (34). L-arginine, but not D-arginine, preserved the Ca2+-dependent NOS activity during ischemia/reperfusion, which correlated with the enhanced recovery in cardiac performance (34). This finding supports the view that the protective effect of L-arginine is due to maintained release of NO synthesized by eNOS during reperfusion (Fig. 1).

The finding that Ca2+-dependent NOS activity was lost during ischemia/reperfusion and that this loss was prevented by administration of L-arginine may indicate that one possible mechanism of reduced Ca2+-dependent NOS activity is lack of substrate. Under basal conditions L-arginine exists in excess (4) but during ischemia and reperfusion this situation may be changed and the availability of L-arginine may become more important for the function of the enzyme. Furthermore, in the absence or suboptimal concentrations of L-arginine, Ca2+-dependent NOS generates superoxide anion and hydrogen peroxide (35, 36), leading to impairment of cellular function. Therefore, supplementation of L-arginine may be essential for NOS to produce NO rather than toxic oxygen free radicals in a situation when the intracellular concentration of L-arginine is rate-limiting for NOS. The observed loss of Ca2+-dependent NOS activity in the vehicle hearts and the preserved Ca2+-dependent NOS activity by L-arginine following ischemia/reperfusion is also in good accordance with the finding that endothelium-dependent vasodilatation by ACh is clearly reduced in vehicle hearts but unchanged in L-arginine treated hearts subjected to ischemia/reperfusion.

No significant iNOS activity has been demonstrated in hearts subjected to short periods of ischemia and reperfusion (30 min ischemia and 30 min reperfusion) (34). In contrast, a significant increase in iNOS activity was reported to occur in the infarcted myocardium 48 h after coronary ligation (37). An increased activity of iNOS leading to cardiotoxic production of NO may explain the protective effect of administration of NOS inhibitors following long periods of reperfusion in the rabbit heart (31-33). Since the expression of iNOS occurs several hours after stimulation, the discrepancy between the obtained results might be due to the different observation times following myocardial ischemia. However, since the major events which lead to reperfusion injury occur during the first minutes of reperfusion (25), the increase in NO production by the possibly increased expression of iNOS activity in the late period of reperfusion would not be expected to contribute to any major extent to the ischemia/reperfusion injury.

There are several potential mechanisms by which the L-arginine/NO pathway can be cardioprotective during ischemia and reperfusion. A first possibility is by maintaining a reduced coronary vascular tone (Fig. 1). It is well known that during reperfusion a part of the myocardium is underperfused which is known as the no-reflow phenomenon. Since NO is a potent coronary vasodilator, loss of endothelial NO during reperfusion may aggravate the no-reflow phenomenon. Accordingly, the no-reflow area of the isolated rat hearts was clearly smaller after pretreatment with L-arginine or the NO donor SNAP (28). A second protective mechanism may be that NO can act as a scavenger of free radicals (Fig. 1). Oxygen-derived free radicals are formed during ischemia and reperfusion both under in vitro and in vivo conditions by various cell types such as neutrophils, myocytes and endothelial cells (38, 39). Since NO produced from L-arginine and NO donors is a free radical itself, it rapidly reacts withe and thereby inactivates other cytotoxic free radicals. A third possible cardioprotective mechanism may be inhibition of leukocytes (Fig. 1). It is well known that a large number of leukocytes accumulate within the jeopardized myocardium during reperfusion. Several studies have also shown that the extent of leukocytes accumulation in the myocardium correlates to the infarct size (40). Since NO inhibits leukocyte aggregation (4) it is likely that reduced NO

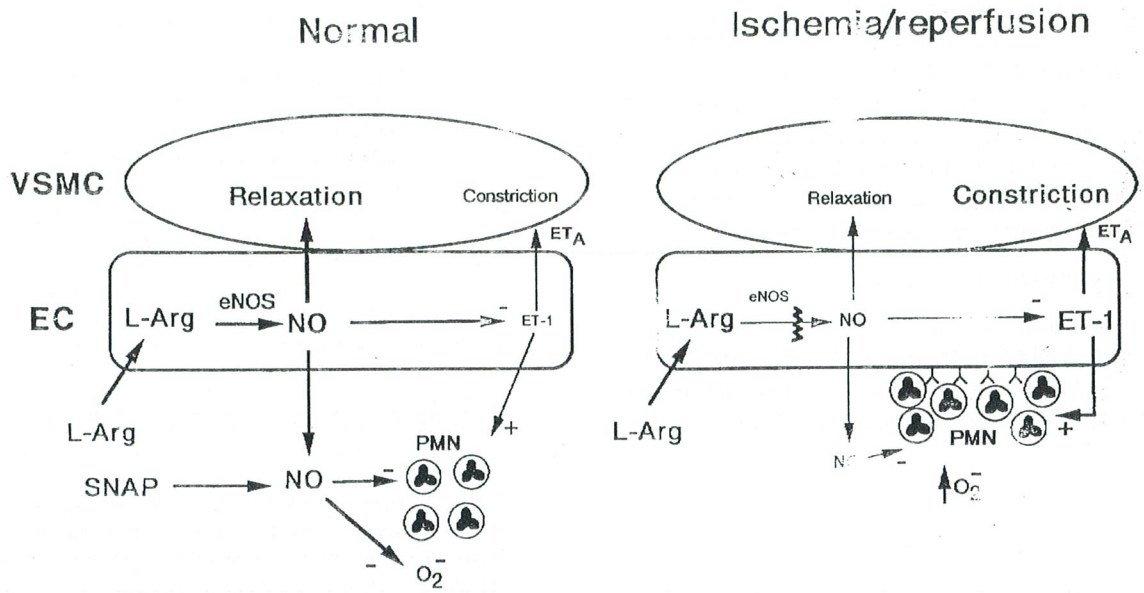

Fig - 1 : Schematic illustration describing the putative importance of the L-arginine (L-Arg)/nitric oxide (NO) pathway during myocardial ischemia and reperfusion. Under normal situations NO produced in endothelial cells (EC) causes relaxation of vascular smooth muscle cells (VSMC), inhibits aggregation of polymorphonuclear leukocytes (PMN) and inactivates oxygen free radicals. Ischemia/reperfusion results in reduced activity of Ca2+-dependent endothelial NO synthase (eNOS) in the myocardium thus reducing NO production. The reduction in NO production may therefore result in an increase in vascular tone, enhanced adhesion of PMN and oxygen-derived free radicals. Furthermore, since NO inhibits production of endothelin-1 (ET-1), loss of NO may result in enhanced production of ET-1 which can activate ETA receptors that further increases vascular tone. Administration of L-Arg and NO donors like SNAP during the course of ischemia/reperfusion in order to maintain NO levels attenuates myocardial and endothelial injury. The protective effect of L-arginine is inhibited by the NOS inhibitor L-NNA and seems to be mediated by preserving the eNOS activity and the release of NO.

production following reperfusion increases the accumulation and infiltration of leukocytes. In in vivo studies it was demonstrated that the cardioprotective effects of L-arginine and NO donors correlated to their inhibition of myeloperoxidase activity (27, 29). On the other hand, the cardioprotection evoked by L-arginine and SNAP in the isolated rat hearts is unlikely to to be secondary to leukocyte inhibition due to the extremely small numbers of cells present in this model. An additional interesting possibility is the interaction between NO and endothelin-1. NO has previously been observed to inhibit production of endothelin-1 (41). In addition, administration of L-arginine inhibits the increase in myocardial endothelin-1 levels observed during ischemia and reperfusion (42). Since endogenous endothelin-1 seems to be involved in the development of ischemia/reperfusion injury (28, 43) part of the cardioprotective actions of NO may be related to inhibition of endothelin-1 production (Fig. 1).

Conclusions

Several cardiovascular diseases including atherosclerosis and myocardial ischemia followed by reperfusion are associated with impaired endothelial function with reduced formation and release of NO. This may result in an imbalance between various endothelial vasoactive factors leading to increased vascular tone, enhanced aggregation of neutrophils and increased accumulation of oxygen-derived free radicals.

Administration of the precursor L-arginine may represent a possibility to anhance endogenous production of NO, thereby inhibiting the progression and of the disease and possibly also prevent tissue injury. The presented results show that administration of L-arginine or an NO donor inhibits the development of ischemia/reperfusion-induced myocardial and endothelial injury. The effect of L-arginine seems to be realated to maintained NO production as revealed by both functional and biochemical data. By supplementation of L-arginine the activity of eNOS and thereby the normal production level of NO will be maintained to evoke cardioprotective effects during ischemia and reperfusion.

Acknowledgements

The authors own work was supported by the Swedish Medical Research Council (10857), the King Gustav and Queen Victoria Foundation and the Swedish Heart and Lung Foundation.

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