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Forum Review

Ischemic Preconditioning Mediated Angiogenic Response in the Heart

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ABSTRACT

Angiogenesis represents a major focus for novel therapeutic approaches to the prevention and treatment of multiple diseases, most notably ischemic cardiovascular disease and cancer. Therapeutic angiogenesis achieved either through the use of discreet angiogenic proteins or by gene therapy is fast emerging as a highly attractive treatment modality for ischemic heart disease. The purpose of this review is to address this important clinical issue through the identification of potential signaling mechanisms by which a short episode of sublethal ischemia known as ischemic preconditioning causes angiogenesis and subsequently improves myocardial salvage following coronary artery occlusion. *Antioxid. Redox Signal.* 6, 413–421.

INTRODUCTION

CORONARY HEART DISEASE is the primary cause of cardiovascular death. After myocardial infarction (MI), there is a progressive myocardial remodeling characterized by left ventricular (LV) dilation, contractile dysfunction, myocyte hypertrophy, and increased matrix protein formation. Ischemic preconditioning (IP) provides the most powerful form of endogenous protection against lethal ischemic injury. The classical preconditioning can be induced by a variety of stimuli other than ischemia. Hypoxia (27, 54, 65), calcium (4), adenosine agonists (56), α_1-adrenergic agents (7), muscarinic agonists (59), and stretch (45) have been used as preconditioning stimuli to induce tolerance of the heart to the subsequent ischemic episode. Short exposure to hypoxia/reoxygenation, either directly or indirectly, produces oxidative stress, which is associated with angiogenesis or neovascularization. This process is thought to be regulated by several growth factors [epidermal growth factor, transforming growth factor-α, β-fibroblast growth factor, vascular endothelial growth factor (VEGF)]. Induction of these angiogenic factors is triggered by various stress responses. For instance, tissue hypoxia exerts its proangiogenic action through various angiogenic factors, the most notable being VEGF, which has been mainly associated with initiating the process of angiogenesis through the recruitment and proliferation of endothelial cells (27). Brief exposure to hypoxia (30–60 min) followed by reoxygenation significantly accelerated (threefold) the rate of tubular morphogenesis. We, as well as others, found that hypoxia followed by reoxygenation, and not hypoxia alone, caused the formation of reactive oxygen species (ROS) and the activation of redox-regulated transcription factor nuclear factor-κB (NFκB), both of which were inhibited by ROS scavengers (28, 29, 52). ROS antagonists inhibited tubular morphogenesis in a dose-dependent manner. In the clinical setting of hypoxia/reoxygenation, enhanced activation of ROS may trigger intracellular signaling that might accelerate neovascularization *in vivo*. In fact, we observed that hypoxic bursts of 5–10 min duration followed by reoxygenation (ischemic or hypoxic preconditioning) led to myocardial angiogenesis in rat myocardial infarction model (18, 53). Use of antioxidant such as dimethylthiourea inhibited preconditioning-mediated myocardial angiogenesis (unpublished observations). Our laboratory has not only shown the production of the hydroxyl ($OH^{\bullet}$). radical during reperfusion of ischemic rat heart, but also demonstrated the production of the $OH^{\bullet}$ radical in the hearts of patients undergoing coronary bypass surgery (10). From the literature, it is documented already that elevated VEGF levels are required for ocular and tumor an-

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giogenesis in animal models. We, as well as others, have documented the existence of a strong correlation between increased VEGF and ischemic hypoxia (28, 29, 52, 53).

IP, like hypoxic preconditioning, not only reduces the extent of infarction, but also causes the salvaged myocardium to have better mechanical function. During the last several years, our laboratory has studied extensively the molecular mechanisms of preconditioning-mediated signal transduction. It was demonstrated that preconditioning-mediated signal transduction cascade triggered by tyrosine kinase and coupled to phospholipase D leads to the activation of mitogen-activated protein (MAP) kinases (36). Additionally, we documented the translocation and activation of the nuclear transcription factor of p38 MAP kinase in IP, resulting in the activation of MAP kinase-activated protein (MAPKAP) kinase 2 (38). A modern experimental strategy for treating myocardial ischemia is to induce neovascularization of the heart by use of angiogens, mediators that induce the formation of blood vessels, or angiogenesis. The process of angiogenesis is regulated by the above-mentioned signal transduction cascade triggered by the signals obtained from the transmembrane receptor tyrosine kinases (RTKs) and nonreceptor tyrosine kinases (Src family) of endothelial cells.

Angiogenesis is known to be the body's natural healing process in which new blood vessels grow in response to injury. Therefore, it is extremely important to develop the body's natural angiogenic process in order to create collateral circulation in areas where blocked coronary arteries deprive the heart muscle of sufficient blood flow, *e.g.,* in the settings of chronic myocardial ischemia. Development of coronary collateral circulation is the heart's own bypass mechanism by which it retains the blood supply to the myocardium at risk (2, 9, 22, 49).

Angiogenic therapy for the human heart is currently being vigorously pursued. In the past 10 years, alternative revascularization/angiogenesis strategies have progressed from bench to bedside, focusing on the capillary sprouting and/or growth of new vessels to replace the old. However, most of the strategies involve the delivery of growth factors. Very little success with these strategies has been demonstrated so far for various reasons. Very recently, we have demonstrated that both hypoxic preconditioning and IP can stimulate myocardial angiogenesis to an extent sufficient to exert significant cardioprotection in a rat model of MI progressing to heart failure as evidenced by increased capillary/arteriolar density and enhanced ventricular contractile functional reserve.

IP MEDIATED ANGIOGENIC RESPONSE IN THE HEART

Stressed myocardium can be adapted to ischemia by repeatedly subjecting it to short-term reversible ischemia, each followed by another short duration of reperfusion. This phenomenon, known as IP, or ischemic adaptation, causes the development of oxidative stress leading to the induction of gene expression, which is subsequently translated, into the production of beneficial proteins responsible for the heart's defense (11, 33).

Substantial evidence exists to support the notion that oxygen-derived free radicals are generated during the reperfusion of ischemic myocardium, resulting in the development of oxidative stress. The adaptive protection has been found to be mediated by gene expression and transcriptional regulation. Our laboratory demonstrated that IP triggers a signaling pathway by potentiating tyrosine kinase phosphorylation (36, 38). Recently, oxygen-derived free radicals have been implicated in the transmembrane signaling process, which is also found to be important in angiogenesis. All stresses, including ischemic stress, have been found to enhance the heart's defense system, as evidenced by increased heat shock proteins (HSPs) and antioxidant enzymes. HSPs are also found to be involved in angiogenic response such as heme oxygenase-1 (HSP 32). IP-mediated angiogenesis and growth factor/receptor stimulation for cardioprotection is a very novel approach and potentially very exciting therapeutic strategy. Preconditioning of heart by repeated ischemia and reperfusion has been found to delay the onset of subsequent irreversible ischemic injury (35). It is well known that preconditioning provides cardioprotection by reducing subsequent postischemic ventricular dysfunction, decreasing incidence of arrhythmias and infarct size. Such myocardial preservation by repeated short-term reversible ischemia leads to the development of the concept of stress adaptation (11). After MI, development of LV enlargement occurs, and this process is known as postinfarction ventricular remodeling. Ventricular remodeling is known to be affected by several factors. One major factor is the infarct size, which is limited by the presence of collateral vessels. Capillary density becomes lower in the border zone than in the remote areas of the infarcted ventricle (14).

Ischemia or coronary artery occlusion has been shown to induce VEGF mRNA in rat hearts (5). VEGF is a well-known endothelial cell-specific angiogenic factor and also a critical regulator of angiogenesis that stimulates proliferation, migration, and proteolytic activity of the endothelial cells (43). An additional report suggested the VEGF-induced expression of Bcl-2, which eventually functions to enhance the survival of endothelial cells in the toxic, oxygen-deficient environment (43). Substantial evidence exists to support the notion that oxygen-derived free radicals are generated during the reperfusion of ischemic myocardium, resulting in the development of oxidative stress (35). Ischemia was found to induce the angiopoietin/Tie receptor system in a focal cerebral ischemia model (31). Myocardial adaptation to ischemic stress in stunned pig myocardium demonstrated the induction of c-jun, c-fos, Egr-1, and jun-B, which may be involved in the repair process of angiogenesis (8). Adenosine is known to limit the degree of vascular injury during ischemia and reperfusion by inhibition of oxygen free radical release, which prevents endothelial cell damage and that might help to preserve endothelial cell function and microvascular perfusion (13). In our ischemic preconditioned rat MI model, we were able to induce angiogenesis after preconditioning. Recently, our study demonstrated that *in vivo* brief repetitive cycles of coronary artery occlusion (5 min) followed by short duration of reperfusion (10 min) triggered myocardial angiogenesis at the capillary and arteriolar levels, which nicely corroborated with the improved myocardial contractile function (18).

INVOLVEMENT OF ANGIOGENIC FACTORS DURING IP

VEGF

The process of angiogenesis is regulated by the signals obtained from the transmembrane RTKs and nonreceptor tyrosine kinases (Src family) of endothelial cells. Flk-1/KDR and Flt-1 are two such RTKs, which together with their ligand VEGF have been shown to control blood vessel development during embryogenesis (17, 62). This receptor/ligand system has been shown to augment neovascularization (3, 62). VEGF is not only an endothelial cell-specific angiogenic factor, but also a critical regulator of angiogenesis that stimulates proliferation, migration, and proteolytic activity of endothelial cells (32). Yet the signaling pathways that modulate the mitogenic effects of VEGF in vascular endothelial cells are still ill defined (32). A recent study demonstrated that VEGF was localized and expressed in the embryonic/fetal heart and that its level remained high during the early postnatal period when capillary proliferation is high (51). It is now well established that alternate exon splicing of a single VEGF gene results in the generation of four different molecular species, having 121, 165, 189, and 206 amino acids following signal sequence cleavage. $VEGF_{165}$ is the predominant molecular species produced by a variety of normal and transformed cells. Transcripts encoding $VEGF_{121}$ and $VEGF_{189}$ are detected in the majority of cells and tissues expressing the VEGF gene (61). Among the mechanisms that have been proposed to participate in the regulation of VEGF gene expression, oxygen tension is a particularly important mediator, both *in vitro* and *in vivo*. VEGF mRNA expression is rapidly and reversibly induced by exposure to low PO_2 in a variety of normal and transformed cultured cell types (39). Also, ischemia caused by occlusion of the left anterior descending coronary artery results in a dramatic increase in VEGF mRNA levels in the pig and rat myocardium, suggesting the possibility that VEGF may mediate the spontaneous revascularization that follows myocardial ischemia (20).

VEGF is a potent vascular endothelial cell-specific mitogen that stimulates endothelial cell proliferation, microvascular permeability, vasodilatation, and angiogenesis (16, 42). VEGF is the only growth factor proven to be specific and critical for blood vessel formation (15). VEGF has also been shown to improve endothelial cell function and survival *in vitro* and vascular reactivity *in vivo* (56). Previous studies demonstrated up-regulation of VEGF mRNA in cardiac tissues by transient ischemia, suggesting that VEGF mediates neovascularization during myocardial ischemia (5, 20). In another recent study, authors documented that IP exerts a cardioprotective effect through nuclear translocation of protein kinase Cε (PKCε) and VEGF-induced angiogenesis (25). We have also determined IP induced VEGF protein expression significantly in an IPMI (ischemic preconditioned myocardium) group when compared with the CMI (nonpreconditionedmyocardium) group 7, 14, and 21 days after MI in the rat survival model. The protein band density of VEGF in the IPMI group increased with each time compared with the CMI group even 21 days after the operation as shown in Fig. 1.

RTKs, Flk-1 and Flt-1, receptors for VEGF

Flt-1 (VEGFR-1) and Flk-1/KDR (VEGFR-2) are the endothelial specific tyrosine kinase receptors of VEGF through which its effects are primarily mediated (12, 62). It has been well established that expression of VEGF, Flt-1, and Flk-1 is up-regulated in response to hypoxia *in vitro* and *in vivo* (39) and to ischemia *in vivo* (30). Studies in a rat MI model demonstrated induction of VEGF (275%), Flk-1 (375%), and Flt-1 (400%) mRNA expression throughout the entire heart after infarction (30). A great deal of attention has been directed toward studies of VEGF expression and its function in myocardial ischemia/hypoxia (18, 33, 37, 44, 52, 53), but relatively little is known regarding the mechanism of its receptors, Flk-1 and Flt-1. VEGF is the only known ligand for Flk-1, whereas Flt-1 is able to bind placental growth factor in addition to VEGF.

Few embryological studies have demonstrated abundance of Flk-1 in the human lung tissues, whereas Flt-1 was found to be abundant in heart, lung, and kidneys (17). Careful investigations demonstrated a functional difference between Flk-1 and Flt-1 in endothelial cells. In the developing human

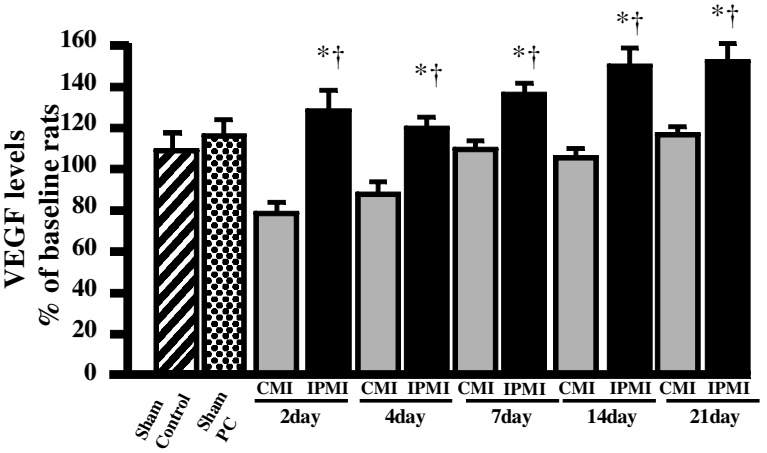

FIG. 1. Results from densitometric scanning of western blots are expressed as percentage mean value of the baseline control (sham) showing the effects of IP followed by LAD occlusion on the expression of VEGF in rat myocardium *in vivo* after sham surgery and 2, 4, 7, 14, and 21 days after LAD occlusion. Similar results were obtained in six independent experiments performed in triplicate. $^{*}p < 0.01$ compared to corresponding sham; $^{\dagger}p < 0.01$ compared to CMI.

heart, both receptors were found to be expressed in the myocardial capillaries, and both were found to stimulate intracellular calcium flux along with VEGF stimulation. Genetically manipulated Flk-1 knockout (homozygous) studies demonstrated early embryonic death due to inhibition of vasculogenesis, whereas in another study homozygous Flt-1 disruption caused failure to assemble normal formation of vascular channels (17). In another important observation, it was found that Flt-1 was expressed in endothelium of both large and small vessels, whereas Flk-1 expression was restricted to small vessels only (30). One of our recent studies demonstrated a distribution pattern of Flk-1 and Flt-1 around the coronary arteries and arterioles that displayed staining along the putative capillaries in a reticular pattern. We have also documented in this study the intensity of staining for both receptors, which increased with increasing durations of hypoxia, but Flt-1 tended to persist, whereas Flk-1 tended to decrease (47). In other words, in the systemic whole body hypoxic rat model, we found significant abundance of Flk-1 in the 1-h hypoxia group, which remained elevated in the 2-h group, but was reduced in the 4-h hypoxia group followed by 24 h of reoxygenation, suggesting a role in the initial rather than later stages of the early angiogenic process. In contrast, induction of Flt-1 protein expression was increased in the 1-h hypoxia group and continued to be elevated even in the 4-h hypoxia group, indicating a more continuous role in the early angiogenic process (47).

We have found very exciting results by using targeted mutations (knockout) of the mouse Flt-1 gene during IP. We demonstrated that inactivation (almost 50%) of the functional mouse Flt-1 gene resulted into impaired postischemic ventricular recovery and increased infarct size in $Flt^{+/-}$ compared with the wild-type (CD-1) control when subjected to IP, suggesting that Flt-$1^{+/-}$ hearts are unable to precondition $Flt^{+/-}$ myocardium to the same extent as wild-type CD-1 control (unpublished observations). Flt-$1^{+/-}$ heterozygous knockout mice also demonstrated significant alteration in the expression of a number of extremely important genes when subjected to ischemia and reperfusion by utilizing a very high technology gene expression profile analysis system such as DNA microarray. The other receptor, Flk-$1^{+/-}$ knockout, demonstrated also a similar effect during preconditioning. These results therefore suggest the involvement of VEGF receptors in IP-mediated cardioprotection.

VEGF-MEDIATED VASCULAR PERMEABILITY IS REDUCED DURING IP

MI leads to persistent postischemic vasogenic edema, which develops as a result of increased vascular permeability (VP). This promotes fluid extravasation and interstitial edema, increased interstitial pressure, collapsing of small vessels, and the loss of perfusion. Hence, reducing VP following MI prevents myocardial damage. Vascular leak caused by a variety of myocardial injuries, including myocardial ischemia, results in myocardial edema, which represents one of the major complications associated with myocardial ischemia. VEGF in response to ischemic injury promotes VP. Previous studies demonstrated that Src kinase regulates VEGF-mediated VP in the brain following injury (46). In our present study, we have determined significant down-regulation of c-Src protein expression in the IPMI heart following MI compared with CMI. Src kinase activity is known to be increased during the acute phase of preconditioning as a result of oxidative stress (41). We showed that inhibition of Src by PP1 attenuated myocardial edema following MI, leading to reduced infarction and increased blood flow. Therefore, inhibition of c-Src activity in conjunction with VEGF overexpression enhances the benefit of this angiogenic growth factor while obviating its potential detrimental effect on myocardial survival and function through an increase in VP (Fig. 2).

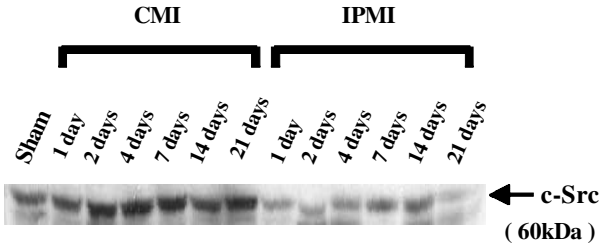

FIG. 2. Representative western blot analysis showing the effect of IP on the expression of c-Src in rat myocardium *in vivo* at 1, 2, 4, 7, 14, and 21 days after MI. c-Src was expressed as a 60-kDa protein (partly reproduced with permission from JMCC; reference 18).

IP MEDIATED INCREASED ENDOTHELIAL AND CARDIOMYOCYTE CELL SERVIBILITY: INVOLVEMENT OF SURVIVAL PROTEINS

Angiogenesis induced by the most promising protein, VEGF, has been found to be associated with enhanced cell survival in human umbilical vein endothelial cells *in vitro* (1, 56). It is well established that adjunctive local injection of VEGF demonstrated great promise for many patients who would not be candidates for any form of revascularization. Currently, it is undergoing clinical trial as both gene therapy and direct administration of protein. The temporal pattern of VEGF, Bcl-2, and survivin expression documented in a study at the protein level by western blot analysis correspond to the histological evidence (data not shown) of angiogenesis in the rat MI model. Our findings suggest that VEGF and Bcl-2 were up-regulated in response to IP after 2 days of MI, and the expression of both proteins stayed high enough significantly at the latter stage of MI (studied up to 21 days) and functioned endogenously to promote myocardial angiogenesis (18). These results complement previous studies in which supplemental VEGF and Bcl-2 *in vitro* and *in vivo* have been shown to induce angiogenesis in various tissues and cell cultures (19), whereas survivin, another antiapoptotic protein downstream of Bcl-2, plays a significant role in the earlier phase than the latter phase of antiapoptotic pathways. The expression of survivin was found to be significant after 2 days of MI in the preconditioned group, increased further compared with the corresponding CMI group, and then slowly decreased and became normal after 7 days.

This result forces us to speculate its role in the earlier phase to protect the myocardium rather than its role in the latter phase of MI.

Recently, a very interesting study demonstrated IP induced translocation of PKCε isoform to the nucleus and enhanced expression of VEGF mRNA in the infarcted cardiomyocytes. This study also documented increased capillary density and reduction of infarct size in the *in vivo* model of rat MI. Use of chelerythine, a specific inhibitor of PKC, inhibited the beneficial effect of IP, thus supporting the relation between PKC activation, VEGF mRNA up-regulation, and increased angiogenesis along with reduced infarct size triggered by IP (25).

ACTIVATION OF TRANSCRIPTION FACTORS IN ISCHEMIC PRECONDITIONED MYOCARDIUM

To explore the mechanism of this beneficial effect of intermittent IP in rat myocardium in the setting of MI, we tried to explore the signal transduction pathway by studying various transcription factors and their involvement with several anti-death factors in relation to cell death. We know that various redox-regulated transcription factors, such as activator protein-1 (AP-1) (6), SP1, and NFκB (63) are known to be regulated and found to be important regulators of angiogenesis. Several studies have shown that AP-1 and NFκB are differentially activated by oxygen tension. It is already documented that hypoxia/reoxygenation, but not hypoxia alone, caused the production of ROS and thereby activated NFκB (28, 29). Consistent with these previous reports, DNA binding activity of NFκB remained almost at the baseline level when rats were subjected to hypoxia only. In contrast, when the rats were subjected to hypoxia followed by reoxygenation, a significant amount of DNA binding activity was observed in the myocardium. This indicates that the angiogenesis is accompanied by and also requires the formation of ROS. This study also provides evidence for direct involvement of ROS and ROS-mediated signaling via NFκB *in vivo* in myocardial angiogenesis. In our experimental condition, inhibition of NFκB by pyrrolidine dithiocarbamate (PDTC) was able to inhibit myocardial capillary density.

Recently, it was demonstrated in a mouse model that NFκB activation is obligatory for retinal angiogenesis, because the administration of PDTC, a NFκB inhibitor, suppressed retinal neovascularization (64). Several potential binding sites for the transcription factors AP-1, AP-2, and SP1 are localized in the VEGF gene promoter, and among eight glioma cell lines, cellular mRNA levels of transcription factors SP1 and AP-1 were found to be closely correlated with those of VEGF. Besides these redox-regulated transcription factors, Stat3, Pax-5, and TFIID were also found to be significantly activated (except AP-1) by protein/DNA array analysis and later validated by gel-shift analysis (Fig. 3) in the IPMI group after 2 days of left anterior descending coronary artery (LAD) occlusion compared with the CMI group. In the ischemic preconditioned group, the proapoptotic transcription factor AP-1 was rightly manipulated and therefore reduced compared with the CMI group, whereas activation of Stat3 in this model demonstrates significant beneficial effect of IP-mediated cardioprotection in the infarcted heart through the induction of the antiapoptotic gene Bcl-2. However, significant activation of Pax-5, which is a key regulator of lineage-specific gene expression and TFIID in the setting of MI, is yet to be explored.

IMPROVED MYOCARDIAL BLOOD FLOW BY IP IN THE SETTING OF CHRONIC MI: INCREASED CAPILLARY AND ARTERIOLAR DENSITY

The results of our study documented that IP can possess angiogenic potential and can improve myocardial blood flow and cardiac function followed by severe ischemic myocardial injury. Therefore, myocardial adaptation to intermittent ischemia appears to be a highly promising approach to induce angiogenesis in a rat model of MI as evidenced by increased capillary and arteriolar density. This increased microvascular growth was found to be associated with a reduced infarct size and significant preservation of contractile functional reserve. Pharmacological cardiac stress testing with dobutamine revealed differences in the extent of cardiac contractile reserve between IPMI and CMI. The ischemic preconditioned group displayed significantly elevated contractile reserve compared with the CMI group. LV contractile reserve measured with dobutamine is a sensitive means of detecting differences in the extent of infarction. The dobutamine stress test is often utilized to detect the presence of hibernating myocardium in clinical chronic myocardial ischemia. Angiogenic effects of IP in the functional improvements and the decreased infarct size are very general. In hibernating myocardium, the myocytes are viable, but cannot contract normally because of the lack of sufficient blood supply. The presence of hibernating myocardium indicates that such a myocardial region is absent from the blood supply through the major coronary arteries, but is rich in collateral blood vessels as a result of enhanced angiogenesis. In our experiment, the dobutamine stress test appears to suggest the presence of larger areas of hibernating myocardium in the ischemic preconditioned heart than in nonpreconditioned heart. In the present study, IP also triggered significant expression of VEGF and increased perfused capillary density along with increased blood flow. Based on these results, it is also reasonable to find smaller infarction in the IP heart, because the presence of collateral arteries allows more myocytes to be alive after the cessation of blood supply by LAD occlusion. However, we cannot ascribe all infarct size-limiting effects of IP to angiogenesis, because direct protective effects on myocytes of IP cannot be eliminated. Although such an effect may be observed early after LAD occlusion, direct effects of IP on myocytes rather than enhanced angiogenesis is a more likely mechanism for early salvage of the myocardium. This may in turn improve contractile function as assessed by LVdP/dtmax (maximum rate of development of LV pressure). Therefore, infarct size-limiting effect of IP via direct salvage of myocytes early after LAD occlusion only negligibly affected contractile performance in a chronic stage. However, when compared after the dobutamine stress test, IP heart showed a greater increase in LVdP/dtmax than non-IP MI hearts, indicative of the pres-

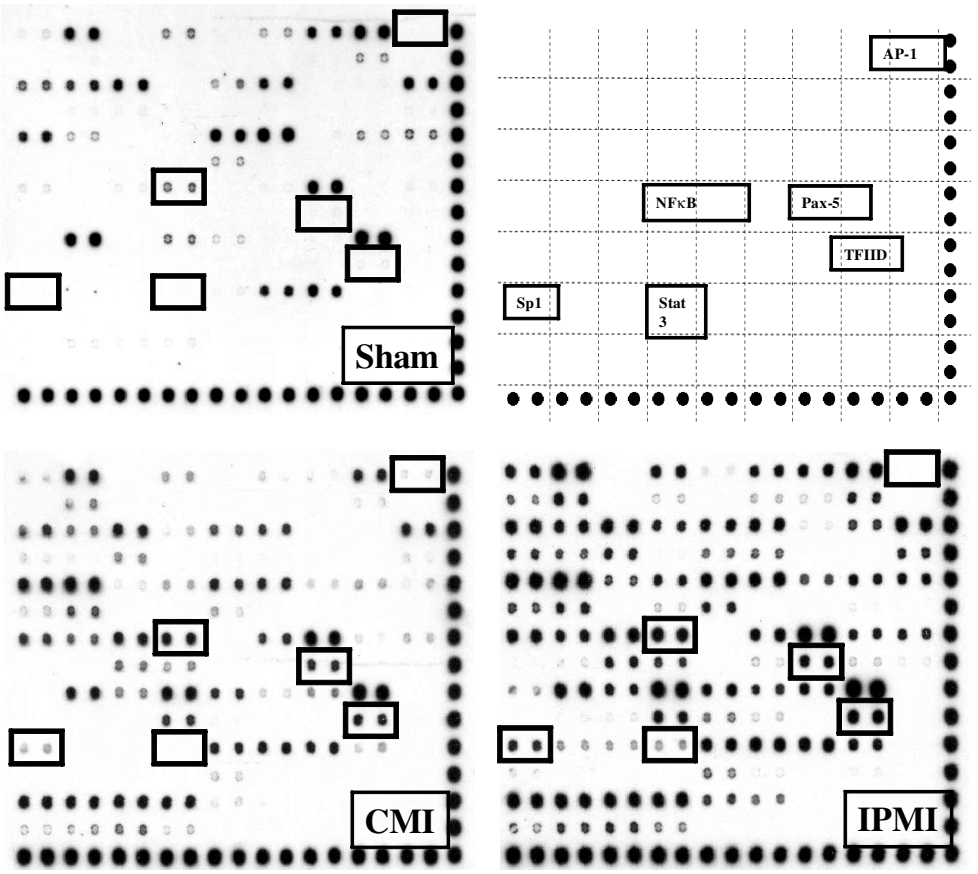

FIG. 3. Representative protein/DNA array analysis demonstrates the activation of several transcription factors after 2 days of post LAD occlusion. Significant activation of Stat3, SP1, AP-1, TFIID, Pax-5, and NFκB were observed in the IPMI group. Sham, control; CMI, nonpreconditioned followed by LAD occlusion; IPMI, ischemic precondition followed by LAD occlusion. (Reproduced with permission from JMCC; reference 18).

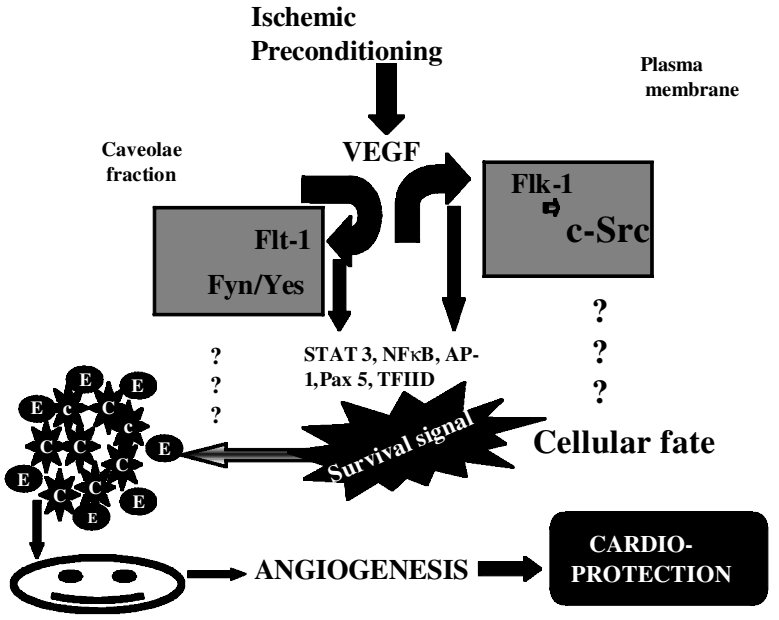

FIG. 4. Possible signaling mechanism during IP-mediated angiogenesis. c, cardiomyocytes; E, endothelial cells.

ence of a larger number of viable but hibernating myocytes being supplied blood flow presumably through newly developed collateral blood vessels in the IP heart. The blood flow data also determined increased blood supply in the IPMI group after 2 days post-op compared with the CMI group in this study, which persisted even after 21 days post-op. A possible signaling pathway that mediates angiogenesis and thereby cardiac protection by IP is shown in Fig. 4.

CONCLUSION

In conclusion, it is logical to propose that reoxygenation preceded by ischemic hypoxia induces a cascade of events initially triggered by ROS involving VEGF and related protein expression to induce myocardial angiogenesis in the rat MI model as evidenced by increased capillary/arteriolar density. Myocardial adaptation to IP appears to be a highly promising approach to reduce cellular injury due to ischemia and reperfusion. Further studies utilizing IP protocols to promote angiogenesis in experimental models of chronic MI and heart failure will help in the characterization and identification of promising target candidates for gene therapy to achieve clinically relevant myocardial angiogenesis.

ACKNOWLEDGMENTS

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ABBREVIATIONS

AP-1, activator protein-1; CMI, nonpreconditioned myocardium; HSP, heat shock protein; IP, ischemic preconditioning; IPMI, preconditioned myocardium; LAD, left anterior descending coronary artery; LV, left ventricle; LVdP/dtmax, maximum rate of development of LV pressure; MAP, mitogen-activated protein; MI, myocardial infarction; NFκB, nuclear factor-κB; PDTC, pyrrolidine dithiocarbamate; PKC, protein kinase C; PP1, (4-amino-5(4-methylphenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine); ROS, reaction oxygen species; RTK, receptor tyrosine kinase; Src, nonreceptor tyrosine kinases; VEGF, vascular endothelial growth factor; VP, vascular permeability.

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