Myocardial Protection by Monophosphoryl Lipid A: Potential Mechanisms

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INTRODUCTION

Monophosphoryl lipid A is a derivative of the lipopolysaccharide (LPS) from gram-negative bacteria (52,53). This drug has had several different abbreviations in the literature, including MPL, MPLA, and the most commonly used—MLA. It was extracted to reduce the associated toxicity while retaining the immunomodulatory properties of the parent endotoxin molecule. MLA has been shown to induce certain beneficial immunostimulatory effects such as macrophage stimulation, cytokine release, and a variety of other effects upon both humoral and cell-mediated immunological host defense response (3,17,21,27,36,61). The beneficial effects of MLA have been used in 1) inducing tolerance to endotoxemia in both laboratory animals and humans, 2) immunotherapy or immunoprophylaxis, 3) adjuvant for a number of vaccines, 4) inducing delayed protection against cerebral vasospasm caused by subarachnoid hemorrhage, and 5) delayed cardioprotection through complex mechanisms. This paper provides a detailed review of the current state of knowledge of the role of this drug in myocardial protection.

CHEMISTRY AND PHARMACOKINETICS

The chemical structure of MLA is shown in Fig. 1. MLA was derived from bacterial LPS of several gram-negative strains that include *Salmonella typhimurium* (53), *Salmonella minnesota R595* (52), and *Chlamydia trachomatis* (51) by removing a phosphoester from reducing sugar of the disaccharide followed by saponification of a long-chain \( \beta \)-hydroxy ester from the 3-position hydroxyl group of reducing glucosamine (52,53). Injection of MLA (5000 \( \mu \)g/kg, i.p.) in rats resulted in a uniform distribution in kidneys, liver, lungs, spleen, and stomach. Its amount decreased significantly by 48 h as compared with...
24-h posttreatment in all of the organs except spleen (55). These results indicate an active elimination process of MLA from these tissues.

**IMMUNOSTIMULATORY EFFECTS**

Similar to endotoxin, MLA can induce a variety of isotypes of cytokines (5,17,21,23,60), including tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), interleukin 12 (IL-12), and interferon-γ (INF-γ), that activate and recruit macrophages as well as inactivate suppressor T cell activity. This process eventually results in an enhancement of the immune response. The immunostimulatory effects of MLA have been exploited to induce tolerance against bacterial infection (9) and endotoxemia (17,26) and as a prophylaxis against sepsis and septic shock (14). Astiz et al. (2) demonstrated that MLA protected against sepsis caused by gram-negative as well as gram-positive bacterial strains. They further showed that MLA could be used as an agent in localized therapy for peritonitis (4).

The tumor-suppressing effects of bacterial endotoxin have been known for years. MLA with its reduced toxicity has been considered as a viable alternative to endotoxin. The antitumor effects of MLA were demonstrated by a number of investigators using both in vitro and in vivo animal models (56,57,70). MLA has also been extensively investigated as a vaccine adjuvant (1,40). It has been shown to be an effective adjuvant for vaccines against several common infectious diseases such as hepatitis B (71), malaria (59,66), genital warts (72), and HIV-1 infection (62). MLA has also been studied as an adjuvant for cancer vaccines (26,39,46,54).

**PHASE I HUMAN TRIALS**

There are at least two published MLA studies that were performed in human subjects. In 1984, Vosika et al. (76) studied the clinical toxicity and immunological effects of MLA.
in 17 cancer patients. MLA derived from either S. typhimurium G30/C21 or S. minnesota R595 was given intravenously to patients twice a week for 4 w for a total of eight doses. Vital signs and other immunological parameters were closely monitored after the drug administration for the first week of therapy. They reported that the doses through 100 μg/m² of body area were well tolerated with minimum side effects. At 100 μg/m², mild fever (<38.5°C), chills, and mild rigor occurred in 29% of cases, whereas at 250 μg/m² chills and rigor occurred in 86% of cases. One patient was treated at a dose of 500 μg/m² and experienced hypotension (blood pressure decreased from 126/72 to 80/40) after the injection. There were no consistent changes in renal or liver function that could be attributed to the drug treatment. A significant decrease in WBC count occurred at 1 h after treatment and returned to normal by 3 h. A similar transient decrease was also found in the platelet count 24 h following the first treatment and returned to normal by 48 h. There were also an increase in total active T cells and a decrease in monocytes in the patients receiving 100 μg/m² over the first day of treatment.

More recently, Astiz et al. (3) investigated the systemic effects of MLA in 44 healthy volunteers. No side effects or increases in cytokine levels were observed in the subjects who received an i.v. dose of MLA lower than 10 μg/kg. Subjects who received a dose of 20 μg/kg experienced a moderate increase in body temperature, heart rate, and release of TNFα, IL-6, and IL-8. All of the symptoms were well tolerated and required no therapy. Furthermore, 24 h after the pretreatment with MLA (20 μg/kg) or vehicle, these subjects were challenged by a bolus of intravenous injection of Ec-5 endotoxin (20 IU/kg). The subjects receiving MLA had significantly less severe systemic symptoms and cytokine releases compared with the vehicle-treated subjects.

**MLA IN CARDIOVASCULAR PROTECTION**

**Antiischemic Effects**

The majority of studies reported a reduction in myocardial infarct size following ischemia/reperfusion in MLA-pretreated dogs (31,49,50,83,85), pigs (87), rabbits (6,12,19, 86,89), rats (64), and mice (80). Figs. 2 and 3 demonstrate representative studies of MLA-induced infarct reduction. In addition, several studies demonstrated that MLA protects the heart against stunning caused by either brief, repetitive episodes of ischemia/reperfusion (84) or a prolonged ischemia (15,29,38,73,88). However, some studies failed to show any significant ventricular functional improvement in MLA-treated rabbits (86) or mice (80), although the infarct size was significantly reduced in the same animals. The protective effects of MLA have also been reported against ischemia-induced ventricular fibrillation and/or ventricular tachycardia in isolated rat heart (15,73) and in vivo rat (64) and rabbit (69) models. Similar protection was also observed in an in vivo rabbit model (69). This antiarrhythmic effect was seen not only in the healthy, anesthetized animals but also in the conscious rabbits with hypercholesterolemia and atherosclerosis (69) 24 h after MLA pretreatment. Rodents appeared to require a much higher drug concentration to exert the cardioprotective effects. The cardioprotective doses of MLA were from at least 300 μg/kg (73) to as much as 5000 μg/kg (38,64) for rats and 350 μg/kg for mice (80), whereas a small dose of 10 to 35 μg/kg was sufficient to induce the protective effects in rabbits (6,12,19,86,89), pigs (87), and dogs (31,49,50,83,85). The exact cause for the
species-related difference in MLA dose-response is not clear. It is possible that rodents may have a lower ischemic tolerance (due to higher myocardial oxygen consumption), a faster drug clearance rate (due to smaller body size), or a different level of immunore-
sponse to MLA, as compared with other species.

As described above, most studies have focused on the delayed phase of cardioprotec-
tion; that is, the protection occurred approximately 24 h following the drug treatment. Yao et al. (83,85) reported that no protection was observed 1 h after MLA treatment. On the other hand, Yoshida et al. (86) found that a delayed phase of cardioprotection existed both 12 h and 24 h after the drug treatment. A more complete time course of myocardial infarct size following a dose by intravenous injection of MLA (35 μg/kg, i.v.) was recently reported by Elliott (11; also Fig. 2), who also showed an “early window” for the antiinfarct protection. These early effects seemed to be short-lived and lasted no more than 30 min after the drug treatment. In contrast, a delayed phase of protection reappeared around 6 h and was sustained until 36 to 48 h after the MLA treatment. The biphasic cardioprotective effect is reminiscent of the profile of ischemic tolerance with ischemic preconditioning (41).

In addition to its protective effect in intact animals, MLA has also been found to protect cultured adult rat (37) and chick (65) ventricular myocytes in vitro. The addition of MLA to the cell culture medium (30, 200, 300 ng/mL) resulted in a delayed protection as indicated by a reduction in intracellular enzyme leakage (37,65) and the number of cells killed (65) when the cells were subjected to chemically simulated “ischemia” 20 to 24 h after the MLA pretreatment. These results suggest that MLA may have direct protective effects on cardiac myocytes that bypassed blood-borne factors, such as macrophage ac-
tivation.

Vascular Protection

The effects of MLA on vascular endothelial and smooth muscle function were first investigated by Yao et al. (83), who found that vascular function in both coronary and femoral arteries was transiently decreased by MLA and returned to nearly normal levels 24 h after treatment. Based on their findings, these authors stated that MLA did not appear
to protect against endothelial or smooth muscle injury caused by ischemia/reperfusion even though the infarct size was reduced 24 h after the treatment. However, a recent study by Richard et al. (58) demonstrated that pretreatment with MLA (450 μg/kg, i.v.) resulted in delayed protection 24 h later against vascular endothelial dysfunction induced by ischemia/reperfusion. As shown in Fig. 4, MLA pretreatment ameliorated the diminished relaxation responses to acetylcholine in the isolated rat coronary arteries following

FIG. 3. Delayed cardiovascular protection induced by MLA in mice. 1) Left graph: myocardial infarct size following 20-min global ischemia and 30-min reperfusion (I/R). Mice were pretreated with MLA 24 h prior to I/R with either a low (35 μg/kg, i.p.; MLA35) or a high (350 μg/kg, i.p.; MLA350) dose. The infarct size was significantly reduced in the MLA350 group. The MLA-induced infarct reduction was abolished by the selective iNOS inhibitor, S-methylisothiourea (SMT; 3 mg/kg, i.p.; 30 min before I/R) and was completely absent in iNOS knockout mice. 2) Upper right graph: MLA350 resulted in an increase of both the pre- and the postischemic coronary flow rate that appears to be independent of iNOS. 3) Lower right graph: Tissue nitrite/nitrate accumulation in nonischemic or ischemic/reperfused mouse hearts pretreated with vehicle or MLA. Twenty-four hours after the pretreatment with high dose MLA, NO production was significantly augmented in the ischemic/reperfused hearts and was associated with the infarct reduction. *, P ≤ 0.05 versus the vehicle group. From ref. 80 with permission.
ischemia/reperfusion. The discrepancy between these studies (58,83) may be due to the different animal species (dog vs. rat) as well as drug doses (65 vs. 450 µg/kg). Interestingly, in our recent study in a Langendorff isolated mouse heart model (80, see also Fig. 3), we observed a significant improvement in both pre- and postischemic coronary flow in the mice pretreated with MLA at a high dose (350 µg/kg) but not at a low dose (35 µg/kg). It seems that the MLA-induced vascular protection is dose dependent as well.

In addition, Toyoda et al. (74) reported that MLA also induced delayed protection in cerebral blood vessels against vasospasm caused by subarachnoid hemorrhage. They demonstrated that the subarachnoid hemorrhage-elicited vascular spasm was significantly attenuated in the rabbits pretreated intrathecally with MLA (5 µg/kg) 24 h prior to the induction of hemorrhage.

**MECHANISMS OF MLA-INDUCED CARDIOVASCULAR PROTECTION**

The mechanisms of MLA-induced protection are not fully understood, although several hypotheses have been suggested. This subject was previously reviewed by Elliott (11, also see Fig. 5). The present article includes the most updated information and results from a number of new publications during 1998–1999. Our discussions on the complex signal transduction cascade are presented in the following two sections: that is, 1) the triggers, mediators, and messengers; and 2) the end-effectors. Our current view on the key pathways of MLA-induced delayed cardiovascular protection is illustrated in Fig. 6.

**Triggers, Messengers, and Mediators**

**Nitric Oxide**

Nitric oxide (NO) plays an important role in signal transduction mechanisms (18) including those involved in the ischemic myocardium (28). NO is one of the key modulators of vascular smooth muscle tone, and its biological action can be cardioprotective against ischemia/reperfusion injury through coronary vasodilatation and reduction in myo-
Cardiac oxygen consumption via cGMP-dependent as well as cGMP-independent mechanisms (18,20,24,35). More recently, NO has been appreciated as the possible trigger and mediator for ischemic preconditioning (7,44). A number of studies in recent years have demonstrated the ability of MLA to induce the inducible nitric oxide synthase (iNOS) in several types of tissues either in vitro or in vivo (16,60,75). In ischemic rabbit myocardium, Zhao et al. (89) demonstrated that delayed cardioprotection with MLA can be abolished by aminoguanidine—an inhibitor of iNOS. It was also shown that the iNOS mRNA expression was upregulated in both rat (73) and pig (87) myocardium. In a recent study (80; Fig. 3), we pretreated adult male ICR mice and B6,129 homozygous (−/−) iNOS knockout mice with MLA. The ischemia/reperfusion protocol was carried out in the Langendorff isolated perfused mouse heart model (79). The protocol consisted of 30 min of stabilization, 20 min of no-flow global ischemia, and 30 min of reperfusion. Parameters of cardiac function as well as myocardial infarct size were assessed in this mouse model. In addition, the NO oxidation products (nitrite and nitrate) that accumulated in the heart tissue homogenate were measured. In this investigation, we observed that the cardioprotection was abolished by S-methylisothiourea (SMT), a selective inhibitor of iNOS (68). Therefore, the abrogation of MLA-induced antiischemic effect could be at least in part due to the inhibition of iNOS in the ischemic heart. Furthermore, the complete lack of MLA-induced protection in the iNOS knockout mice suggests an obligatory role of this isoform...
of NOS in the protective process. We also found that the cardioprotective dose of MLA significantly increased NO production following ischemia/reperfusion but not in the non-ischemic hearts, suggesting that iNOS was functional only in the ischemic hearts (80). Similar results were reported previously in MLA-treated rabbit (89) and rat (15) hearts. These data suggest that posttranslational modifications of the iNOS enzyme are required before it is capable of generating NO (43). It is possible that ischemia may activate certain kinases (such as protein kinase C (PKC) and tyrosine kinase) or inhibit phosphatases that may promote phosphorylation-dependent activation of the inactive iNOS induced by MLA within certain time periods after drug administration. Indeed, Ping et al. (45) demonstrated that NO generated by ischemic preconditioning or NO donors caused translocation of the epsilon isoform of PKC that leads to a downstream signaling cascade in

FIG. 6. Mechanisms of MLA-induced delayed myocardial protection. The cardioprotection may be triggered by generation of free radicals and/or cytokine release, which in turn activates transcription factors such as nuclear factor kappa B (NFκB) and activator protein 1 (AP-1). The nuclear translocation of these transcription factors may upregulate iNOS gene expression and consequently open the sarcolemmal and/or mitochondrial K$_{ATP}$ channels through the signaling or other direct physiological actions of NO via cGMP-dependent as well as -independent pathways.
delayed preconditioning. In addition, NO by itself or through the second messenger cGMP could also modulate K\textsubscript{ATP} channels. The cGMP-dependent protein kinases may be capable of phosphorylating K\textsubscript{ATP} channels and priming the channel leading to cardioprotection (13).

The role of constitutive forms of NOS (cNOS) in MLA-induced protection is still unclear. There is a good possibility that MLA may also activate cNOS, which may serve as the trigger for secondary induction of iNOS. In our studies, we observed a consistent improvement in preischemic coronary flow in all MLA-treated groups. Blocking iNOS with SMT did not reverse the improvement of coronary flow in MLA-treated mice. These data suggest that MLA may have been improving vascular endothelial function independently of the iNOS enzyme.

**Adenosine and Bradykinin Receptors**

Most of the studies on the role of adenosine- in MLA-induced protection were performed by Przyklenk et al. (49,50) in a canine model. They found the disparate effects of MLA pretreatment and ischemic preconditioning on myocardial adenosine levels and the activity of an adenosine-regulating enzyme—5\textsuperscript{'}-nucleotidase (5\textsuperscript{'}-NT) (49). There was no correlation between 5\textsuperscript{'}-NT activity and infarct reduction. In fact, during both of the preconditioning approaches, the myocardial 5\textsuperscript{'}-NT activity and adenosine level were not elevated above the control level in order to achieve infarct reduction. Taken together, an adenosine-dependent mechanism for the MLA-induced cardioprotection is unlikely to be the primary determinant. In addition, Mazenot et al. (30) recently demonstrated that pretreatment with MLA (100 µg/kg, i.v.) in rabbits did not induce bradykinin B\textsubscript{1} receptor synthesis and the associated hypotensive response to (des-Arg\textsuperscript{9})-bradykinin 24 h later. The result indicated that the MLA-induced delayed cardioprotection did not appear to be related to a B\textsubscript{1} receptor-dependent pathway. However, the exact role of bradykinin receptors in mediating MLA-induced protection needs to be further examined by using B\textsubscript{1} or B\textsubscript{2} antagonists and/or gene knockout mice.

**Calcium**

There has been increasing recognition for a pivotal role played by intracellular calcium (Ca\textsuperscript{2+}) in mediating the phenomenon of preconditioning. Recent studies from several groups suggest that calcium serves as a key messenger for activating a PKC-dependent signal transduction pathway in both ischemic and calcium-induced preconditioning (32, 34,48). The role of calcium in MLA-induced cardioprotection is unknown. A recent report by Proksch et al. (47) demonstrated that lipid A is able to induce a rapid rise in intracellular Ca\textsuperscript{2+} concentration in the intact rat renal proximal tubules through the release of intracellular Ca\textsuperscript{2+} stores. Therefore, further studies are warranted to examine whether calcium is also involved in the drug-induced protection in ischemic heart, especially concerning its role in activating the PKC pathway and/or the calcium-dependent nitric oxide synthase.

**Free Radicals**

Free radicals such as reactive oxygen species play a crucial role in intracellular signal transduction mechanisms (63) and in ischemic preconditioning. Delayed preconditioning
effects were abolished by pretreatment with free radical scavengers in both conscious pigs (67) and isolated rat myocytes (90). Very recently, Das et al. (10) confirmed these findings in isolated rat hearts and further proposed that the free radical signaling mechanism of preconditioning is independent of PKC although potentiated by tyrosine kinase phosphorylation, resulting in the activation of mitogen-activated protein (MAP) kinases and transcription factors. The exact role of free radicals in the underlying mechanisms of delayed cardioprotection with MLA has not yet been well defined. Maulik et al. (29) provided indirect evidence for a role of free radicals in triggering the drug-induced late cardioprotection by demonstrating increased lipid peroxidation in the rat hearts treated with LPS or MLA (500 μg/kg, i.p).

**Cytokines**

It is well known that MLA stimulates cytokine release in various cell types (5,17,21, 23,61), except in one rabbit study that showed a lack of TNFα induction with a cardioprotective dose (35 μg/kg, i.v.) of MLA (12). The role of cytokines in MLA-induced delayed cardioprotection has not been directly investigated. In order to determine the role of cytokines, it is necessary to examine a number of other isotypes of cytokine that may be involved in different drug doses as well as animal species. The signal transduction actions of cytokines may be undertaken by activating their own receptors on cell membrane and/or by generating free radicals that in turn activate the cytosolic transcription factors that lead to modification of gene expression in certain effector proteins that are responsible for the MLA-induced antiischemic protection.

**Transcription Factors**

It is quite clear that generation of free radicals and/or cytokines by bacterial LPS can activate transcription factors, such as nuclear factor kappa B (NFκB) and/or activator protein 1 (AP-1), which seem to be the key intermediate factors of the signal transduction cascade (63). Recently, Xuan et al. (82) demonstrated that NFκB serves as a common downstream mediator that converts the multiple signals elicited by ischemic stress to induction of gene expression of certain protective proteins in the heart, for example, iNOS. Considering the similarity of LPS and MLA in both chemical structure and biological effects such as inducing free radicals and cytokines, it is logical to assume that these transcription factors may also be involved with the MLA-induced late cardioprotection.

**End-Effectors**

**Induction of Cytoprotective Proteins**

Induction of cardioprotective proteins by preconditioning has been the subject of investigation during the past decades (41). Brown et al. (8) demonstrated that LPS-induced cardioprotection was associated with a significant increase in endogenous catalase activity. A plausible explanation for the delayed cardioprotection induced by MLA is that this drug may induce synthesis of cytoprotective proteins, such as antioxidant enzymes. In fact, Nelson et al. (38) found an association of postischemic functional improvement with a significant increase in catalase enzyme activity in rat hearts. It should be noted that the
drug dose used by this study was very high (5000 μg/kg, i.p.). An upregulation of basal superoxide dismutase (SOD) activity was also found in the basilar arteries of MLA-treated rabbits that manifested less cerebral vasospasm caused by hemorrhage (74). In contrast, Yao et al. (84) found only a marginal increase in myocardial catalase activity and no changes in SOD activity in MLA-treated dogs (35 μg/kg, i.v.) as compared with the control group. These studies suggest that antioxidants may not be the major end-effector(s) in the cardioprotective effect of MLA.

Heat shock proteins (HSPs) were also considered as the potential end-effector protein of delayed preconditioning (41). It has been shown that pretreatment with LPS induced 70 kDa heat shock protein (HSP70) expression in rat myocardium that was associated with delayed cardioprotection (33). However, the cardioprotective dose of MLA failed to induce HSP70 in the intact rabbit heart (6,86). In contrast, in vitro exposure of cultured cardiac myocytes with MLA demonstrated increased expression of HSP70 that was associated with significant reduction in the release of creatine kinase (CK) and lactate dehydrogenase (LDH) following simulated ischemia (37). It is possible that the MLA (200 ng/ml) used to precondition myocytes may have induced oxidative stress, leading to the expression of HSP70, and that in vivo protection with MLA is mediated by another endogenous mediator that may not be involved with induction of HSP70.

Inhibition of Neutrophil Infiltration

Neutrophil infiltration contributes to myocardial ischemia/reperfusion injury (25). Yao et al. (83,85) first proposed that MLA-induced reduction in myocardial infarction may have been, at least in part, due to attenuated neutrophil infiltration into the ischemic border zone, the site of the most active inflammatory response. These findings were confirmed by Zhao et al. (89) in an in situ rabbit model. Interestingly, the iNOS inhibitor, amino-guanidine, partially blocked the attenuated infiltration of neutrophils in MLA-treated animals, suggesting a role of NO in these studies. Indeed, the concept is supported by a previous report of Pabla et al. (42), who showed that exogenous NO attenuates neutrophil-mediated myocardial contractile dysfunction after ischemia and reperfusion. However, the NO-mediated neutrophil inhibition may not be the sole end-effector for the MLA-induced cardioprotection, since MLA pretreatment also protected the isolated buffer-perfused hearts subjected to global ischemia/reperfusion (29,38,73,80) despite the virtual absence of neutrophils in the ischemic myocardium.

Opening of ATP-sensitive Potassium (K<sub>ATP</sub>) Channels

Several studies suggest the involvement of K<sub>ATP</sub> channels in the mechanism of ischemic preconditioning (13) and pharmacological protection with MLA (12,19,31), since the cardioprotection can be abolished by either glibenclamide or 5-hydroxydecanoate (5-HD), the blockers of sarcolemmal and/or mitochondrial K<sub>ATP</sub> channels. Opening of the K<sub>ATP</sub> channel appears to be protective because of the increase in outward potassium current, resulting in shortening of the action potential, which in turn may spare ATP and thereby allow entry of less calcium into the myocyte through the voltage-sensitive calcium channel. Decreased intracellular calcium overload may reduce ischemic injury and lead to better myocyte preservation. Recently, Stambaugh et al. (65) demonstrated that MLA-
induced late cytoprotection in cultured ventricular myocytes was blocked by glibenclamide, 5-HD, and a nonspecific NOS inhibitor, N-monomethyl-L-arginine (L-NMMA). They also found that MLA-induced late protection was additive to the early protective effects of the agonists of adenosine A1 and A3 receptors and of a KATP channel opener, possibly acting at the level of the KATP channel. Taken together, as illustrated in both Fig. 5 and Fig. 6, the mechanism of MLA-induced delayed cardioprotection appears to include induction/activation of nitric oxide synthase with subsequent NO-mediated opening of myocardial KATP channels (35).

SUMMARY AND FUTURE PERSPECTIVES

The detoxified derivative of bacterial endotoxin, MLA, is a potent immunostimulant and is also able to induce significant late cardiovascular protection against myocardial ischemia/reperfusion injury. This antiischemic effect is associated with enhanced NO production in the ischemic tissue and can be blocked by selective inhibition of iNOS. Furthermore, the direct cause-and-effect relationship of NO in MLA-induced protection is provided by our recent studies that demonstrate the absence of an antiischemic effect of this drug in iNOS knockout mice. In addition, the increased NO production as a result of iNOS activation may open the sarcolemmal and/or mitochondrial KATP channels that appear to be the end-effectors of the drug-induced cardioprotection. Despite a great deal of evidence supporting iNOS as the obligatory mediator and KATP channels as the possible end-effector, the upstream signal transduction including the initial triggering mechanism remains largely unknown. The multifactorial mechanism may involve free radicals, cytokines, transcription factors, and tyrosine/protein kinases that appear to be interchangeably related or linked.

Further investigations would be able to elucidate the following: 1) the potential role of endothelial nitric oxide synthase (eNOS) or neuronal nitric oxide synthase (nNOS) in MLA-induced cardioprotection; 2) the specific cytokine(s) and their receptors that may be responsible for activation of iNOS; 3) a potential role of free radicals in activating PKC and tyrosine kinases that lead to further downstream signaling through phosphorylation of the effector proteins; 4) a possible role of calcium in activating PKC and/or calcium-dependent eNOS or nNOS; and 5) other pharmacological agents that could induce late preconditioning via similar signal transduction pathways as MLA. For example, we have recently observed that RC552 (a synthetic glycolipid compound with even fewer side effects than MLA) mimics the chemical structure of MLA and can also induce INOS-dependent delayed cardioprotection in mice (81); similarly, a few other investigators have also reported their preliminary results showing RC552-induced protection in dogs (22, 77, 78).

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REFERENCES


