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Effect of Amrinone, a Selective Inhibitor of Phosphodiesterase III, on PMNs-induced Cardiac Dysfunction in Ischemia/reperfusion

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Ischemia followed by reperfusion in the presence of polymorphonuclear leukocytes (PMNs) results in a marked cardiac contractile dysfunction. Amrinone, a specific inhibitor of phosphodiesterase 3, has an antioxidant activity against PMNs. Therefore, we hypothesized that amrinone could attenuate PMNs-induced cardiac dysfunction by suppression of reactive oxygen species (ROS) produced fby PMNs. In the present study, we examined the effects of amrinone on isolated ischemic (20 min) and reperfused (45 min) rat hearts perfused with PMNs. Amrinone at 25 μM, given to hearts during the first 5 min of reperfusion, significantly improved coronary flow, left ventricular developed pressure ($P<0.001$), and the maximal rate of development of left ventricular developed pressure ($P<0.001$), compared with ischemic/reperfused hearts perfused with PMNs in the absence of amrinone. In addition, amrinone significantly reduced myeloperoxidase activity by 50.8%, indicating decreased PMNs infiltration ($p<0.001$). Superoxide radical and hydrogen peroxide production were also significantly reduced in fMLP- and PMA-stimulated PMNs pretreated with amrinone. Hydroxyl radical was scavenged by amrinone. fMLP-induced elevation of $[Ca^{2+}]_i$ was also inhibited by amrinone. These results provide evidence that amrinone can significantly attenuate PMN-induced cardiac contractile dysfunction in the ischemic/reperfused rat heart via attenuation of PMNs infiltration into the myocardium and suppression of ROS release by PMNs.

Key Words: Amrinone, Ischemia, Polymorphonuclear leukocyte, Reperfusion,

INTRODUCTION

Early reperfusion of the ischemic myocardium plays an important role in minimizing myocardial tissue injury associated with acute myocardial infarction. Recently, cardiologists have devised techniques to open the occluded (i.e., thrombosed, stenosed, plaque filled) coronary arteries using either administration of thrombolytic agents (e.g., streptokinase, tissue plasminogen activator, or urokinase), or by surgical interventions (e.g., angioplasty). The purposes of these approaches are to induce a "reperfusion" of previously occluded coronary artery and effectively reestablish flow in the ischemic myocardium. However, reperfusion displaces the sludge accumulated in the occluded coronary artery and washes it downstream into the coronary microcirculation, producing a form of "reperfusion injury" (Braunwald & Kloner, 1985). Reperfusion injury is also an inflammatory event initially due to endothelial dysfunction and eventually leads to neutrophil infiltration into the affected myocardium (Tsao et al, 1990; Entman et al, 1991). The primary mechanism of the augmented myocardial injury resulting from abrupt reperfusion is to release humoral mediators from the neutrophils including proteases, free radicals, and cytokines (Lefer & Lefer, 1993, 1996).

The dipyridine-type phosphodiesterase inhibitor, amrinone is a newly developed noncatechol, nonglycoside drug with combined positive inotropic and vasodilating properties. Amrinone and milrinone are selective inhibitors of cyclic GMP inhibitable phosphodiesterase or phosphodiesterase 3 (Beavo & Reifsnyder, 1990). Drugs of this type enhance myocardial contractility by preventing the breakdown of cyclic AMP in cardiac myocytes (Silver et al, 1988) and cause vasorelaxation due to inhibition of the breakdown of both cyclic AMP and cyclic GMP in vascular smooth muscle (Murray, 1990; Walsh et al, 1995). Increased cyclic AMP in myocardium increases muscle contractions by increasing calcium available from the sarcoplasmic reticulum. In vascular smooth muscle cells, on the other hand, cyclic AMP decreases intracellular calcium an increasing calcium resequestration into the sarcoplasmic reticulum, thus producing relaxation and vasodilation. It has been demonstrated that amrinone and milrinone decrease infarct size in rabbit hearts with coronary artery branch occlusion (Rump et al, 1993a, 1994, 1995), which has been attributed to a vasodilator action resulting in increased myocardial perfusion in the tissue surrounding the ischemic zone (Rump et al, 1994). The reduction in ischemic damage by amrinone in isolated rabbit hearts was not

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ABBREVIATIONS: AMP, adenosine monophosphate; GMP, guanosine monophosphate; PMN, polymorphonuclear leukocyte.

mimicked by the β-adrenoceptor agonist isoprenaline, which also elevates cyclic AMP levels (Rump et al, 1993b). This finding suggests that increased tissue levels of cyclic AMP cannot explain the actions of amrinone and milrinone in ischemia-reperfusion, and that these agents may have additional pharmacological actions. For example, in rat heart and isolated atria, milrinone decreased β-adrenoceptor induced tachycardia (Minatoguchi & Majewski, 1991), opposite to what would be expected of phosphodiesterase inhibition as β-adrenoceptor induced tachycardia is mediated by cyclic AMP. Furthermore, milrinone and to a greater extent amrinone have a very different profile of action compared to other phosphodiesterase 3 inhibitors in that they produce a larger degree of vasodilatation relative to their inotropic effects (Taira, 1987). The question arises whether amrinone and milrinone may possess other pharmacological actions independent of phosphodiesterase inhibition. Our data showed that amrinone inhibited superoxide and hydroxyl radical formated by PMNs.

We reasoned that amrinone could attenuate PMNs mediated injury in a leukocyte-dependent isolated perfused rat heart model of ischemia and reperfusion in which no previous studies have been performed. Therefore, the major purpose of this study was to investigate whether amrinone was able to protect from cardiac contractile dysfunction, and PMNs accumulation associated with ischemia reperfusion injury in a carefully controlled model of rat myocardial ischemia/ reperfusion was dependent upon PMNs to mediate the cardiac contractile dysfunction.

METHODS

Chemicals

Amrinone, N-Formylmethionylleucylphenylalanine (fMLP), phorbol 12-myristate 13-acetate (PMA), sodium heparin, phenol red, horseradish peroxidase, hydrogen peroxide, type II oyster glycogen, citrate phosphate dextrose, hexadecyltrimethyl ammonium bromide, o-dianisidine, and fura-2/AM were all purchased from Sigma Chemical Co. (St. Louis, MO, USA.). Other chemicals were of analytical grade.

Isolated rat heart experiment

Sprague-Dawley male rats (250 ~ 300 g) were anesthetized with 40 mg/kg sodium pentobarbital and administered 1,000 U sodium heparin i.p. Following a midline thoracotomy, the hearts were rapidly excised, the ascending aorta was cannulated, and retrograde perfusion of the heart was initiated on a Langendorff apparatus at a constant pressure of 80 mmHg. These isolated hearts were perfused with a Krebs bicarbonate buffer of the following composition (in mmol/liter): glucose, 17; NaCl, 120; $NaHCO_3$, 25; $CaCl_2$, 2.5; EDTA, 5.9; and $MgCl_2$, 1.2, and maintained at 37°C. The perfusate was oxygenated with 95% O_2 + 5% CO_2 which were equilibrated at pH 7.3 to 7.4. Two sidearms in the perfusion line located just proximal to the heart inflow cannula allowed infusion of PMN and plasma directly into the coronary inflow line. To asses cardiac contractile function, a 2.5 Fr microtip catheter transducer (Millar Instruments, Inc.) was inserted directly into the left ventricular cavity as previously reported (Pabla et al, 1996; Lefer et al, 1997). Left ventricular pressures, maximal rate of development of left ventricular pressure (+dP/dt min), coronary flow, and heart rate were all recorded using a PowerLab data acquisition system (ADI Diagnostics Inc.) in conjunction with an IBM computer. All data were stored and analyzed at the end of each experiment.

Isolation of rat neutrophil

Neutrophil donor rats (300 ~ 350 g) were injected which 0.5% 10 ml glycogen. Eighteen hours later, the rats were anesthetized with ethyl ether and the PMNs were harvested by peritoneal lavage in PBS. The peritoneal lavage was centrifuged at 3,000 rpm and 4°C for 10 min, as described (Lefer et al, 1997). Finally, the PMNs were washed in Krebs buffer and counted using a microscope. These neutrophil preparations were $>95\%$ pure, and $>95\%$ viable by 0.3% trypan blue as the criterion for viability. Furthermore, PMNs obtained by this method have been found to respond normally in cell adhesion tests (Lefer et al, 1997).

Preparation of rat plasma

Whole blood was obtained by performing an intracardiac puncture in anesthetized rats with a plastic syringe (20 ml) with a 20 gauge needle containing 2.0 ml of sodium citrate-phosphate-dextrose solution. The whole blood was immediately spun in a refrigerated centrifuge at 3,000 rpm for 10 min, and the plasma was decanted.

Experimental protocol of perfused heart

After 15 min stabilization period, baseline left ventricular developed pressure (LVDP), +dP/dt max. and coronary flow were measured every 5 min for 15 min to ensure complete equilibration of the hearts. Flow of Kreb's buffer was then reduced to zero, creating global, total ischemia. This ischemia was maintained for 20 min. The flow was then restored to that of pre-ischemic level and reperfusion of the heart was initiated. At reperfusion, 100×10^6 PMNs and 5 ml of plasma was infused directly into the coronary circulation over a period of 5 min via a set of side ports situated just proximally to the heart in the perfusion line. The PMNs were suspended in 5 ml of Kreb's buffer in a syringe. The plasma was also placed in a syringe located just proximal to the inflow port to the coronary circulation. The hearts were allowed to reperfuse for a total of 45 minutes during which time data were collected every 5 minutes for the first 30 minutes and at the 45 minutes time point. The amrinone was dissolved in plasma at a concentration of 25 μM and infused with the over the first 5 minutes of reperfusion. The dose was determined as an effective cardioprotective dose.

Determination of cardiac tissue myeloperoxidase

Myocardial tissue myeloperoxidase (MPO), occurs virtually exclusively in PMNs (Mullane et al, 1985), and therefore, an increased cardiac MPO activity indicates a significant accumulation of PMNs in the myocardium. MPO was determined spectrophotometrically by the method of Bradley et al. (Bradley et al, 1985) as modified by Mullane et al. (Mullane et al, 1985), and the assays were performed without knowledge of the group from which each sample originated. One unit of MPO is defined as that quantity

of enzyme to hydrolyze 1 mmol of peroxide per minute at 25°C.

Measurement of superoxide anion production

The superoxide anion produced was assayed by superoxide dismutase-inhibitable reduction of ferricytochrome c. The reaction mixtures (200 μl) in 96 well microplate contained 3×10^5 PMNs, 75 μM ferricytochrome c, stimulating agent and DMEM, pH 7.4 and were placed at 5% CO_2 incubator for 4 h at 37°C. Absorbance was measured in a microplate reader (Molecular Devices, Spectra MAX 340, Molecular Devices, Co., Sunnyvale, CA, U.S.A.). The amount of reduced ferricytochrome c was represented as nM using the extinction coefficient of 2.1×10^4 M^{-1} cm^{-1} at 550 nm (Pick & Mizell, 1981; Markert et al, 1984).

Measurement of hydrogen peroxide production

PMNs (3×10^5 cells/well) were incubated in 200 μl of DMEM containing 0.1 mg/ml phenol red and 0.2 mg/ml horseradish peroxidase for 4 h at 37°C. The reaction was terminated by adding 20 μl of 1 N NaOH, and absorbance was measured at 610 nm (Pick & Mizelll, 1981). The concentration of hydrogen peroxide was calculated using hydrogen peroxide solution as the standard.

Measurement of 2-α deoxyribose degradation

Decomposing effect of amrinone on hydroxyl radicals was determined by the assay of malondialdehyde chromogen formation, resulting from 2-α deoxyribose degradation (Aruoma, 1994; Halliwell & Gutteridge, 1999). The reaction mixtures contained 2 mM 2-α deoxyribose, 50 μM $FeCl_3$, 50 μM EDTA, 500 μM H_2O_2, 100 μM ascorbate, 150 mM KCl and 50 mM NaH_2PO_4 buffer, pH 7.4 in a final volume of 1.0 ml. After 30 min of incubation, the reaction was stopped by adding 1 ml of 1% thiobarbituric acid in 50 mM NaOH and 1 ml of 2.8% trichloroacetic acid. Absorbance was measured at 532 nm.

Assay of cytosolic free calcium

Fura-2 loading and fluorescence measurement were performed by the method of Luscinskas et al. (1990). PMNs (approximately 5×10^7 cells/ml) were loaded with 2 mM fura-2/AM to 1 μM/10^7 cells at 37°C for 10 min in the reaction mixtures which contained Hanks' balanced salt solution (HBSS) buffer without calcium and magnesium (HBSS-CMF) and 20 mM HEPES-tris, pH 7.4. The suspension was then diluted 5 fold with 0.5% bovine serum albumin containing HBSS-CMF and further incubated at 37°C for 15 min. After loading, the suspension was centrifuged at 200 g for 10 min, and PMNs were resuspended in 0.1% bovine serum albumin containing HBSS-CMF. This procedure was repeated once more. PMNs were finally suspended in bovine serum albumin free, HBSS-CMF as approximately 5×10^7 cells/ml. Fluorescence measurement was done with Aminco-Bowman Series 2 spectrometer (SLM Instrument Inc. USA.). Preloaded PMNs (4×10^6) were suspended in 1.23 mM calcium and 1 mM magnesium containing HBSS in a final volume of 1 ml. After preincubation at 37°C for 5 min with amrinone, the response was initiated by the addition of 1 μM fMLP. The fluorescence change was read at an excitation wavelength of 340 nm and emission wavelength of 505 nm.

Statistical analysis

All data are presented as mean±SEM. Data were compared by ANOVA using post-hoc analysis with Fisher's corrected test. The data on coronary flow and left ventricular function were analyzed by ANOVA incorporating repeated measures. Probability values of 0.05 or less were considered to be statistically significant.

RESULTS

Experimental protocol

To determine whether amrinone can attenuate leukocyte-endothelial interactions and improve cardiac contractile function in a well characterized model of myocardial ischemia reperfusion, we perfused rat hearts at control flow for 80 min, or for 15 min of control flow followed by 20 min of total global ischemia and 45 min of reperfusion at control flow in the presence or absence of PMNs and plasma. Perfusion of rat hearts with 25 μM amrinone was performed in control hearts at a perfusion pressure of 80 mmHg for 80 min. Perfusion with amrinone at 80 mmHg during sham ischemia or during ischemia/reperfusion without PMNs resulted in no change in coronary flow (CF), left ventricular developed pressure (LVDP), or the first derivative of LVDP (+dP/dt), indicating that amrinone did not exert any direct effects on cardiodynamics (Figs. 1~3). Also, perfusion of non-ischemic hearts with PMNs did not alter any of the cardiac function variables measured, indicating that PMNs do not induce cardiac dysfunction in normal non-ischemic hearts. There was a marked reduction in cardiac contractile function and coronary flow only in ischemic reperfused rat hearts perfused with PMNs.

Coronary flow maintained at zero during ischemia showed a recovery of 78±3% of control in the presence of PMNs

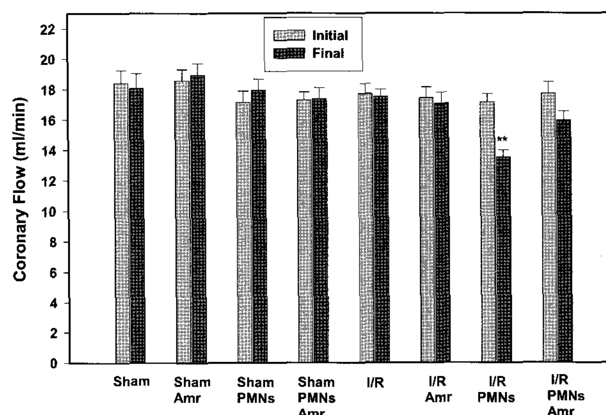

Fig. 1. Effect of amrinone on initial and final coronary flow. Data are expressed in ml/min in the isolated perfused rat hearts subjected to global total ischemia for 20 min and 45 min of reperfusion. Ischemic hearts were perfused in the persence or absence of PMNs (100 million). All values are expressed as mean± SEM for seven individual experiments. Amr; 25 μM amrinone. **P <0.01.

(Fig. 1) However, in the presence of amrinone, coronary flow showed a recovery of 90±4% with the same number of PMNs under the same conditions (Fig. 1).

Changes in left ventricular function (LVDP) showed the pattern similar to that of coronary flow. In ischemic reperfused rat heart perfused with PMNs, LVDP only partially recovered following reperfusion stabilizing at a deficit in LVDP of 50±4% ($p<0.001$) (Fig. 2). However, under the same I/R condition in hearts perfused with the same number of PMNs, amrinone showed markedly significant cardioprotective effect in LVDP, as shown by a 88±3% recovery of initial values.

The first derivative of LVDP (+dP/dt max) also showed the pattern similar to those of coronary flow and LVDP. There was a 46±5% reduction in the final +dP/dt max in untreated PMNs perfused hearts subject to I/R ($p<0.001$) (Fig. 3). However, ischemic/reperfused hearts given amrinone showed a hightly significant preservation of cardiac contractility, comparable to that of control values (i.e., 81±4%).

In all of the controls and non-PMNs perfused I/R heart, cardiac myeloperoxidase (MPO) activity could not be detected, indicating that in nonischemic hearts or in I/R hearts without addition of PMNs there are very few resident PMNs. However, I/R hearts perfused with PMNs showed a highly significant MPO activity, signifying PMNs accumulation ($p<0.001$). Furthermore, when these ischemic/reperfused hearts were perfused with PMNs and given amrinone after reperfusion, a significantly decreased myocardial MPO activity was observed, indicating decreased PMNs infiltration ($p<0.001$) (Fig. 4). This reflects an antineutrophil effect of amrinone.

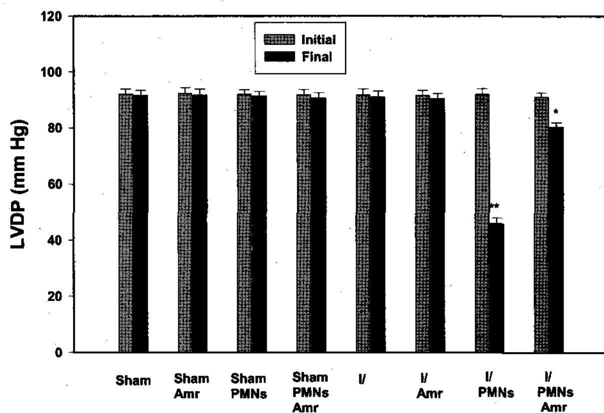

Fig. 2. Effect of amrinone on initial and final ventricular developed pressure (LVDP) in the isolated perfused rat hearts prior to ischemia and following reperfusion. Ischemic hearts were perfused in the persence or absence of PMNs. PMNs caused a marked contractile dysfunction which was attenuated by 25 μM amrinone (Amr). All values are expressed as mean±SEM for seven individual experiments. *P<0.05, ***P<0.001.

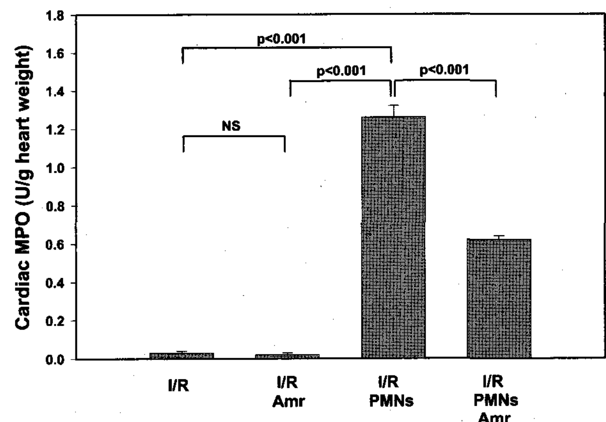

Fig. 4. Cardiac myeloperoxidase (MPO) activity in cardiac sample obtained from ischemic reperfused rat hearts either in the presence or absence of PMNs and 25 μM amrinone. Amrinone (Amr) significantly attenuated the increase of MPO in ischemic perfused hearts with PMNs. MPO activity is expressed in units per gram of wet tissue weight. All values are expressed as mean±SEM for seven individual experiments.

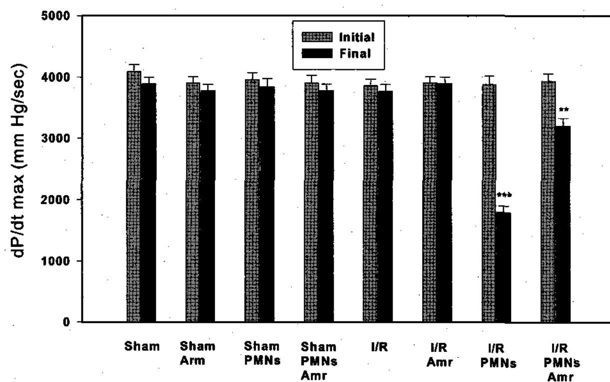

Fig. 3. Effect of derivative LVDP (+dP/dt max) on rat hearts subjected to ischemia and reperfusion. Ishemic hearts were perfused in the presence of PMNs. PMNs caused a significant impairment which was eliminated by 25 μM amrinone (Amr). All values are expressed as mean±SEM for seven individual experiments. **P<0.01, ***P<0.001.

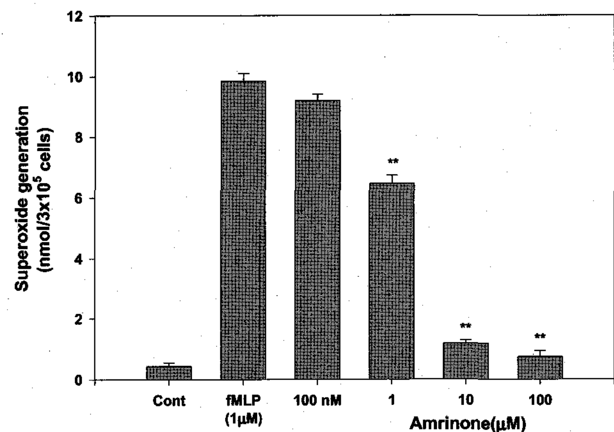

Fig. 5. Inhibition of fMLP-induced superoxide production by amrinone. PMNs (3×10^5 cells) were treated with 1 μM fMLP in the presence of variable concentrations of amrinone. Values are means±SEM, n=5. **P<0.01.

Effects of amrinone on reactive oxygen species

The data in the present study indicate significant beneficial cardioprotective effects of amrinone in a Langendorf perfused heart model of myocardial ischemia/reperfusion, which is dependent upon PMNs to mediate the cardiac contractile dysfunction. fMLP and PMA have been shown to significantly stimulate superoxide and hydrogen peroxide production (Han et al, 1997). Therefore the effects of amrinone on superoxide and hydrogen peroxide production in fMLP- and PMA-stimulated PMNs were examined. One μM fMLP- and 0.1 μg/ml PMA-stimulated PMNs produced 9.84±0.26 (n=5) and 12.85±0.52 (n=5) nmol/3×10^5 cells of superoxide anion, resperctively. Superoxide production in 1 μM fMLP- or 0.1 μg/ml PMA-activated PMNs was inhibited by amrinone in a dose dependent fashion, and 88.1% and 20% of inhibition were observed at 10 μM amrinone, respectively (Figs. 5 and 6). One μM fMLP- and 0.1 μg/ml PMA-stimulated PMNs produced 17.43±0.52 (n=5) and 26.20±0.85 (n=5) nmol/3×10^5 cells of hydrogen peroxide, resperctively. Hydrogen peroxide production in 1 μM fMLP- or 0.1 μg/ml PMA-activated PMNs was inhibited by amrinone, and 42.9% and 48.7% of inhibition was observed at 10 μM amrinone, respectively (Figs. 7 and 8).

Autooxidation of iron liberates reactive oxygen species, and iron causes formation of hydroxyl radical and iron-oxygen complexes. In the present study, hydroxyl radical produced was measured with TBA reactivity of 2-α deoxyribose. As seen in Fig. 9, the increased TBA reactivity of 2-α deoxyribose was decreased by in the presence of 1, 10, and 100 μM amrinone.

Effect of amrinone on calcium mobilization

The cytosolic calcium level was assayed by measuring fluorescence change of fura-2 resulting from the complex

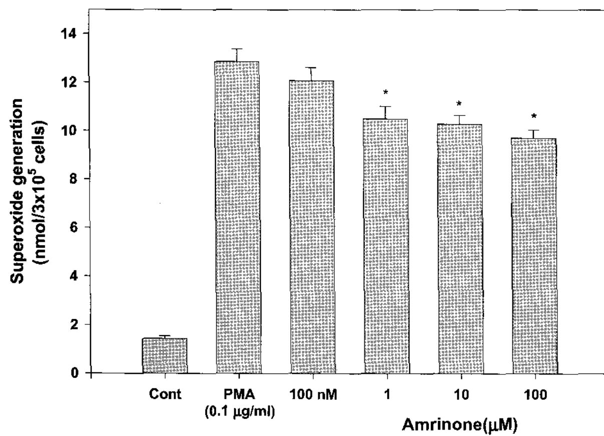

Fig. 6. Inhibition of PMA-induced superoxide production by amrinone. PMNs (3×10^5 cells) were treated with 0.1 μg/ml PMA in the presence of various concentrations of amrinone. Values are means±SEM, n=5. *$P<0.05$.

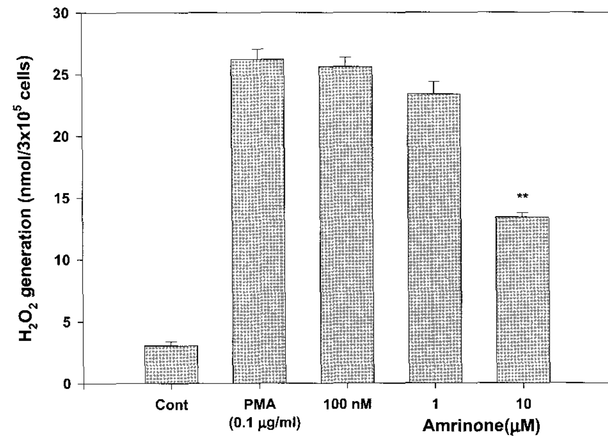

Fig. 8. Effect of amrinone on PMA-induced hydrogen peroxide production. Hydrogen peroxide generated by 0.1 μg/ml PMA-activated PMNs (3×10^5 cells) in the presence of variable concentration of amrinone. Values are means±SEM, n=5. **$P<0.01$.

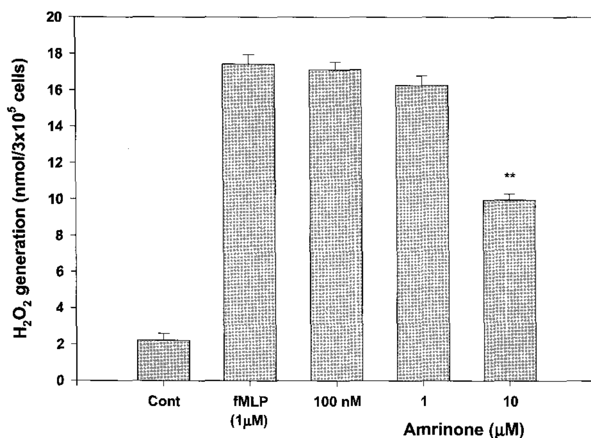

Fig. 7. Effect of amrinone on fMLP-induced hydrogen peroxide production. Hydrogen peroxide generated by 1 μM fMLP-activated PMNs (3×10^5 cells) in the presence of variable concentration of amrinone. Values are means±SEM, n=5. **$P<0.01$.

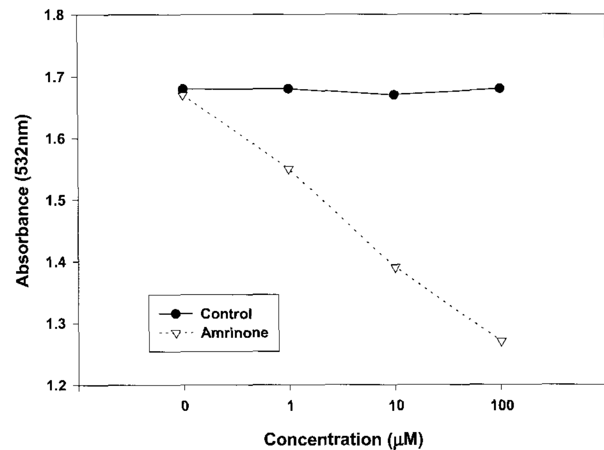

Fig. 9. Effects of amrinone on the 2-α deoxyribose degradation by Fe^{++} and H_2O_2. 2-α deoxyribose (2 mM) was incubated with 50 μM $FeCl_3$, and 500 μM H_2O_2 in the presence of various concentrations of amrinone. Values are absorbance changes and mean, of n=5.

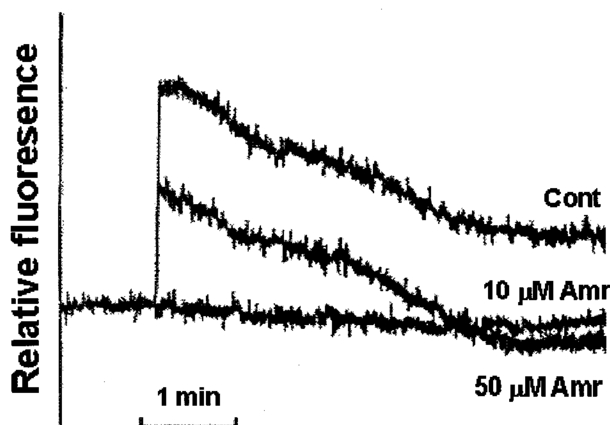

Fig. 10. Effects of amrinone on fMLP-induced elevation of $[Ca^{2+}]_i$. Fura-2 loaded neutrophils (4×10^6 cells/ml) were preincubated with amrinone (Amr) or not (Cont) for 5 min, and the response was initiated by 1 μM fMLP. The traces are representatives of three experiments.

formation of fura-2 and calcium. One μM fMLP elicited an increase of intracellular calcium ($[Ca^{2+}]_i$) in PMNs. The maximum mobilization of $[Ca^{2+}]_i$ occurred within $0.1 \sim 0.2$ sec post addition, and then the level of $[Ca^{2+}]_i$ was gradually decreased. Role of phosphodiesterase 3 inhibitor, amrinone, in fMLP-induced intracellular calcium mobilization was examined. Fig. 10 shows that fMLP-induced elevation of $[Ca^{2+}]_i$ was inhibited by 10 μM amrinone, and 50 μM amrinone completely inhibited fMLP-induced elevation of $[Ca^{2+}]_i$.

DISCUSSION

In the present study, the influence of amrinone on ischemia/reperfusion injury in a Langendorf perfused rat heart model was examined. The data suggest that amrinone exerts a cardioprotective effect on ischemia/reperfusion injury in a carefully controlled model of rat myocardial ischemia/ reperfusion which is dependent upon PMNs to mediate the cardiac contractile dysfunction. This cardioprotection was characterized by a significant maintenance of post-reperfusion coronary flow, left ventricular developed pressure, and the first derivatives of left ventricular pressure (i.e., dP/dt max), indicating a significant attenuation of cardiac dysfunction by amrinone. Amrinone (25 μM) did not seem to exert the cardioprotective effect by directly influencing hemodynamics (i.e., inducing coronary flow or increased cardiac contractility), because amrinone did not alter the functional cardiac index of reperfusion of non-ischemic heart.

Since myocardial ischemia/reperfusion injury has been shown to be related to PMNs infiltrating into ischemic cardial tissue (Tsao et al, 1990; Weyrich et al, 1993), one very important component of the protection afforded by amrinone is its inhibition of PMN accumulation in the ischemic myocardium. Approximately 50.8% attenuation of cardiac MPO activity were oberved in amrinone treated ischemic-reperfused hearts, as compared to those I/R hearts given only the vehicle. This appears to be the key cardioprotective effects of amrinone. Without amrinone, PMNs adhere to the endothelium of the vasculature and release cytotoxic substances such as proteases, eicosanoids, cytokines, and oxygen-derived free radicals (Weiss, 1989), each of which can mediate tissue injury and exacerbate endothelial dysfunction. These humoral mediators have been found to lead to coronary endothelial injury, disruption of the endothelial basement membranes, PMN extravasation, and myocardial necrosis (Weiss, 1989; Lefer et al, 1991; Entman & Smith, 1994). Our finding of reduced MPO accumulation supports the concept that amrinone attenuates PMN-endothelial cell interactions.

Previous studies have shown that administration of recombinant human superoxide dismutase (hSOD) can attenuate coronary endothelial and cardiac contractile dysfunction in isolated perfused hearts (Gillespie et al, 1986: Semb et al, 1989; Hansen, 1995). Superoxide radicals contribute to coronary endothelial dysfunction by inactivation of endothelium-derived nitric oxide (NO) (Patel et al, 1991). Preservation of basal NO release from the coronary endothelium can minimize PMNs adherence to the coronary endothelium, leading to preservation of cardiac contractile function in reperfusion injury (Ma et al, 1993; Lefer & Lefer, 1996). In contrast, decreased basal release of NO promotes PMNs adherence to the coronary endothelium and subsequent transmigration into inflamed tissue (Ma et al, 1991; Ma et al, 1993). After transmigration, further PMN-derived superoxide can damage cardiac myocytes by lipid peroxidation (Nathan et al, 1989; Hansen, 1995). Inhibition of superxide release from PMNs is associated with decreased adherence to the endothelium as well as diminished transmigration into postischemic tissue (Knall et al, 1997; Sue et al, 1997; Mine et al, 1998). In this regard, previous studies have shown that oxygen free radicals upregulate endothelial cell adhesion molecules (e.g., P-selectin) on endothelial ceells and quench endogenous NO released from the endothelium (Patel et al, 1991). NO has been shown to act as a physiological inhibitor of leukocyte-endothelial cell interaction by suppressing up-regulation of endothelial cell adhesion molecules (Lefer & Lefer, 1996). These findings suggest that compounds that suppress superoxide release from PMNs after ischemia and reperfusion may be cardioprotective in PMNs-induced reperfusion injury (Murohara et al, 1995).

Dihydropyridines have been shown to have antioxidant property (Mak & Weglicki, 1990; Weglicki et al, 1990). Elevation of intracellular cyclic adenosine monophosphate (cAMP) in neutrophils is considered to inhibit neutrophil functions, including chemotaxis (Stephens & Snyderman, 1982), respiratory burst (Nielson, 1987) and lysosomal enzyme release (Lad et al, 1985).

One of the possible mechanisms of the cardioprotective effect of amrinone is the inhibition of superoxide and hydrogen peroxide release from infiltrated PMNs. Consistent with this hypothesis, 10 μM amrinone significantly reduced superoxide and hydrogen peroxide production from suspensions of fMLP- and PMA-stimulated rat PMNs ($P < 0.01$) (Figs. $5 \sim 8$).

Another component of the cardioprotective effect of amrinone may invole scavenge of hydroxyl radicals. Supporting this concept, a significant reduction in the increased TBA reactivity of 2-α deoxyribose was observed by 1, 10, and 100 α M amrinone (Fig. 9).

An increase of cytosolic calcium appears to be involved in superoxide production and degranulation (Painter et al, 1984). In order to explore mechanisms by which amrinone

exerts a inhibitory action on free radical production by activated PMNs, effect of amrinone on intracellular calcium mobilization in fMLP-activated PMNs was investigated. Amrinone decreased fMLP-induced intracellular calcium mobilization in PMNs. Its depressant effect on intracellular calcium mobilization by PMNs was in agreement with previously reported data (Cronstein, 1994).

In summary, out results are the first to show a cardioprotective effect of amrinone on PMNs-induced myocardial ischemia/reperfusion injury in the isolated perfused rat heart. These cardioprotective effects appear to be due to inhibition of PMNs superoxide production, hydrogen peroxide generation, and PMNs adherence to the vascular endothelium, and be related to scavenger of hydroxyl radical resulting in fewer PMNs infiltrating into cardiac tissue. These effects would result in less reactive oxygen species release in the cardiomyocytes, thus leading to diminished cardiodepressant effects of PMNs.

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