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Malondialdehyde inhibits cardiac contractile function in ventricular myocytes *via* a p38 mitogen-activated protein kinase-dependent mechanism

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1   Increased oxidative stress plays a significant role in the etiology of cardiovascular disease. Lipid peroxidation, initiated in the presence of hydroxy radicals resulting in the production of malondialdehyde, directly produces oxidative stress. This study was designed to examine the direct impact of malondialdehyde on ventricular contractile function at the single cardiac myocyte level. Ventricular myocytes from adult rat hearts were stimulated to contract at 0.5 Hz, and mechanical and intracellular Ca^{2+} properties were evaluated using an IonOptix Myocam® system. Contractile properties analyzed included peak shortening amplitude (PS), time-to-PS (TPS), time-to-90% relengthening (TR_{90}), maximal velocity of shortening/relengthening ($\pm dLdt$), and Ca^{2+}-induced intracellular Ca^{2+} fluorescence release (CICR) and intracellular Ca^{2+} decay (τ). p38 mitogen-activated protein (MAP) kinase phosphorylation was assessed with Western blot.

2   Our results indicated that malondialdehyde directly depressed PS, $\pm dLdt$ and CICR in a concentration-dependent manner and shortened TPS without affecting TR_{90} and τ. Interestingly, the malondialdehyde-induced cardiac mechanical effect was abolished by both the p38 MAP kinase inhibitor SB203580 (1 and 10 μM) and the antioxidant vitamin C (100 μM). Western blot analysis confirmed direct phosphorylation of p38 MAP kinase by malondialdehyde.

3   These findings revealed a novel role of malondialdehyde and p38 MAP kinase in lipid peroxidation and oxidative stress-associated cardiac dysfunction.

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Abbreviations: CICR, Ca^{2+}-induced intracellular Ca^{2+} fluorescence release; $\pm dLdt$, maximal velocity of shortening/relengthening; MAP, p38 mitogen-activated protein; MDA, malondialdehyde; PS, peak shortening; ROS, reactive oxygen species; TPS, time-to-PS; TR_{90}, time-to-90% relengthening; τ, intracellular Ca^{2+} decay rate

Introduction

Oxidation of polyunsaturated fatty acids of membranes is a common process in the living organism, since polyunsaturated fatty acids in the sarcolemma and sarcoplasmic reticulum are extremely vulnerable to the oxygen-derived free radicals produced during mitochondrial electron transport, which initiates lipid peroxidation (Porter *et al.*, 1995). Hydroxy radicals extract an electron from polyunsaturated lipids generating high-energy lipid compounds. Lipid radicals then react with oxygen to form lipid peroxy radicals ($LOO^{\bullet}$) and lipid peroxide (LOOH). The peroxy radicals react and transfer electrons with neighboring lipids initiating a chain reaction that destroys the integrity of the membrane. Lipid peroxidation affects membrane permeability and alters membrane-bound enzymes/ion channels, which disturbs ion transport and can lead to Ca^{2+} overload (Kaneko *et al.*, 1994; Dhalla *et al.*, 1996; Buffon *et al.*, 2000). Lipid peroxidation has been implicated in the pathogenesis of cardiovascular diseases such as heart failure and atherosclerosis (Holvoet *et al.*, 1995; Diaz-Velez *et al.*, 1996). Both enzymatic and nonenzymatic antioxidant mechanisms including α-tocopherol, ascorbate (vitamin C), uric acid, catalase, superoxide dismutase (SOD) and glutathione peroxidase have been demonstrated to protect against the deleterious effects of free radicals and lipid peroxidation. Nevertheless, the precise nature involving lipid peroxidation-related ventricular dysfunction or the antioxidant-elicited protection against heart failure has not been elucidated.

Malondialdehyde is a terminal product of lipid peroxidation, which can be measured in plasma and serves as an

effective lipid peroxidation marker and an indirect index of reactive oxygen species (ROS) activity (Gutteridge *et al*., 1990). Although enhanced malondialdehyde levels have been reported in patients with compromised heart condition (Diaz-Velez *et al*., 1996), its direct impact on the ventricular function has been masked somewhat by the fact that other concomitant factors such as enhanced production of free fatty acids (FFAs) or harmful FFA metabolites (Hendrickson *et al*., 1997), localized cytokine secretion (interacting with NO synthase) (Katz *et al*., 1994; Torre-Aminone *et al*., 1996) and altered protein metabolism (Berlett *et al*., 1997; Kooy *et al*., 1997) appear to play a role in decreased myocardial contractility and/or intracellular Ca^{2+} homeostasis. Therefore, the aim of this present study is to elucidate the role of malondialdehyde on cardiac contractile function at the cellular level by evaluating myocyte shortening and intracellular Ca^{2+} properties in isolated rat ventricular myocytes.

Methods

Isolation of ventricular myocytes

The experimental procedures described in this study were approved by the institutional animal care and use committee of University of North Dakota (Grand Forks, ND, U.S.A.). Single ventricular myocytes were isolated from adult male Sprague–Dawley rats (200–225 g) as described previously (Wold *et al*., 2002). Briefly, hearts were rapidly removed and perfused (at 37°C) with oxygenated (5% CO_2–95% O_2) Krebs–Henseleit bicarbonate (KHB) buffer (mm: NaCl 118, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, *N*-[2-hydro-ethyl]-piperazine-*N'*-[2-ethanesulfonic acid] (HEPES) 10, glucose 11.1, pH 7.4). Hearts were subsequently perfused with a nominally Ca^{2+}-free KHB buffer for 2–3 min followed by a 20 min perfusion with Ca^{2+}-free KHB containing 223 U/ml^{-1} type II collagenase (Worthington Biochemical Corporation, Freehold, NJ, U.S.A.) and 0.1 mg/ml^{-1} hyaluronidase (Sigma Chemical, St Louis, MO, U.S.A.). After perfusion, the left ventricle was removed, minced and further digested with trypsin (Sigma) before being filtered through a nylon mesh (300 μm) and collected by centrifugation. Cells were initially washed with Ca^{2+}-free KHB buffer to remove remnant enzyme and extracellular Ca^{2+} was added incrementally back to 1.25 mm.

Myocyte shortening and relengthening

Mechanical properties of ventricular myocytes were assessed by an IonOptix Myocam system (IonOptix Inc., Milton, MA, U.S.A.). Cells were placed in a chamber mounted on the stage of an inverted microscope and superfused (at 25°C) with a buffer containing (in mm): 131 NaCl, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, at pH 7.4. The cells were field stimulated at a frequency of 0.5 Hz. Cell shortening and relengthening were assessed using the following indices: peak shortening (PS), time-to-90% PS (TPS) and time-to-90% relengthening (TR_{90}), maximal velocities of shortening ($+$dLdt) and relengthening ($-$dLdt) (Wold *et al*., 2002). To test the effect of malondialdehyde on cardiac contraction, cell shortening was recorded before and 5 min after malondialdehyde application at concentrations 10^{-9}–10^{-3} M) encompassing the

expected physiological range. In some experiments, the antioxidant vitamin C (100 μM,) or p38 mitogen-activated protein (MAP) kinase inhibitor SB203580 (1 and 10 μM) was present prior to and during malondialdehyde application to the myocytes.

Intracellular Ca^{2+} fluorescence measurement

Myocytes were loaded with fura-2/AM (0.5 μM) for 10 min and fluorescence measurements were recorded with a dual-excitation fluorescence photomultiplier system (Ionoptix) as described (Wold *et al*., 2002). Myocytes were plated on glass cover slips on an Olympus IX-70 inverted microscope and imaged through a Fluor $\times$ 40 oil objective. Cells were exposed to light emitted by a 75 W lamp and passed through either a 360 or a 380 nm filter (bandwidths were $\pm$ 15 nm), while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected between 480 and 520 nm after first illuminating cells at 360 nm for 0.5 s then at 380 nm for the duration of the recording protocol (333 Hz sampling rate). The 360 nm excitation scan was repeated at the end of the protocol. Qualitative changes in intracellular Ca^{2+} levels were inferred from the ratio of the fluorescence intensity at two wavelengths (360*380^{-1}) and were presented as changes of fura-2 fluorescence intensity (ΔFFI). Intracellular Ca^{2+} removal was evaluated as the rate of fluorescence decay (τ).

Immunoblot analysis of p38 MAP kinase phosphorylation

To determine phosphorylation of p38 MAP kinase, immunoblot analysis was performed using the method as described by Pollock *et al*. (1993). Briefly, cell lysates of cardiac myocyte (25 μg) were electrophoresed on 10% denaturing sodium dodecyl sulfate polyacrylamide gels. The proteins were transferred electrophoretically to polyvinylidene difluoride (PVDF) membrane (Gelman Sciences, Pierce, Ann Arbor, MI, U.S.A.). Nonspecific binding sites on the membrane were blocked with 5% nonfat dry milk in Tris-HCl containing 0.2% Tween-20 buffer (TBST) overnight at 4°C. The membranes were then probed with phospho-p-38-MAP kinase antibody (1 : 2000; Santa Cruz Biotech, Santa Cruz, CA, U.S.A.) for 1 h at room temperature. After five washings in TBST, the membranes were incubated with the secondary antibody (1 : 4000) for 1 h at room temperature. Finally, membranes were washed three times with TBST. The specific proteins were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ, U.S.A.). The blots were analyzed using Un-Scan-It software to estimate the density of the bands in pixels. The positive control used was NIH -3T3 WCL.

Data analysis

Data were presented as mean $\pm$ s.e.m. Statistical significance ($P < 0.05$) for each variable was estimated by analysis of variance (ANOVA).

Results

Effect of malondialdehyde on myocyte shortening (PS)

The average cell length used in this study was $103 \pm 4 \, \mu M$ ($n = 73$). Acute exposure of malondialdehyde did not affect resting myocyte cell length over the concentration range tested. A representative trace depicting the effect of malondialdehyde (10^{-5} M) on myocyte shortening (PS) is shown in Figure 1a. At the end of a 5 min exposure to this concentration of malondialdehyde, PS was inhibited by 44.6%. Malondialdehyde (10^{-9}–10^{-3} M) elicited a concentration-dependent decrease in PS, with a maximal inhibition of 54.7%. The threshold of response was between 10^{-8} and 10^{-7} M (Figure 1b). The malondialdehyde-induced inhibition in cell shortening was maximal within 4 min of exposure, maintainable up to 20 min and reversible upon a 5 min washout (data not shown). The inhibitory effect of malondialdehyde on myocyte contractility was associated with reduced maximal velocity of shortening/relengthening ($\pm$ dLdt), although the threshold of depression was between 10^{-8} and 10^{-7} M. The duration of shortening (TPS) but not that of relengthening (TR$_{90}$) was shortened by malondialdehyde (Table 1).

Effect of malondialdehyde on intracellular Ca^{2+} transients

To determine whether malondialdehyde-induced inhibition of PS was due to reduced availability of intracellular Ca^{2+}, the influence of malondialdehyde on electrically stimulated increases in intracellular Ca^{2+} (ΔFFI) was examined. Representative traces of intracellular Ca^{2+} transients shown in Figure 2a depict that malondialdehyde (10^{-5} M) significantly depressed ΔFFI. Bar graphs in Figure 2b further revealed that malondialdehyde elicited a concentration-dependent (10^{-9}–10^{-3} M) inhibition on ΔFFI, with a threshold between 10^{-7} and 10^{-5} M. The difference in the threshold between malondialdehyde-induced inhibition of myocyte shortening and that of intracellular Ca^{2+} transients suggests possible involvement of Ca^{2+}-independent mechanisms. In addition, resting intracellular Ca^{2+} levels and intracellular Ca^{2+} decay rate were unaffected by malondialdehyde (Table 2).

Effect of malondialdehyde on myocyte shortening in the presence of the antioxidant vitamin C or the p38 MAP kinase inhibitor SB203580

Lipid peroxidation often deteriorates cellular oxidative damage and may trigger activation of MAP kinase (Leonarduzzi *et al.*, 2000). To examine the role of oxidative stress and the involvement of MAP kinase signaling pathway in malondialdehyde-induced cardiac depression, the effect of malondialdehyde on myocyte shortening was re-examined in the presence of either the antioxidant vitamin C ($100 \, \mu M$) or the p38 MAP kinase inhibitor SB203580 (1 and $10 \, \mu M$). Vitamin C or SB203580 alone had no effect on cell shortening over 30 min (Wold *et al.*, 2002). Interestingly, as shown in Figure 3, the malondialdehyde-induced depression in PS was completely abolished by either vitamin C or SB203580, suggesting involvements of oxidative stress and MAP kinase activation in malondialdehyde-induced cardiac contractile depression.

Effect of malondialdehyde on p38 MAP kinase activation in the absence and presence of the p38 MAP kinase inhibitor SB203580

Our result suggested that p38 MAP kinase may be involved in malondialdehyde-induced cardiac depression. To examine if malondialdehyde directly activates p38 MAP kinase, Western blot analysis was performed to determine the phosphorylation of p38 MAP kinase in response to malondialdehyde (10^{-4} M). Result in Figure 4 exhibits that malondialdehyde directly enhanced phosphorylation of p38 MAP kinase, which can be blocked by either 1 or $10 \, \mu M$ of SB203580. These data

Table 2 Effect of malondialdehyde (MDA) on baseline intracellular Ca^{2+} levels and intracellular Ca^{2+} decay in myocytes from adult rat hearts

	Baseline intracellular Ca^{2+} ($360*380^{-1}$ ratio)	Intracellular Ca^{2+} decay (ms)
Control	1.10 ± 0.01	448 ± 14
MDA 10^{-9} M	1.11 ± 0.01	489 ± 12
MDA 10^{-7} M	1.10 ± 0.01	478 ± 17
MDA 10^{-5} M	1.09 ± 0.01	478 ± 17
MDA 10^{-3} M	1.08 ± 0.01	504 ± 15

Data represent mean $\pm$ s.e.m., $n = 28$–29 cells.

Table 1 Effect of malondialdehyde (MDA) on duration (TPS and TR$_{90}$) and maximal velocity ($\pm$ dLdt) of myocyte shortening and relengthening in the absence and presence of the p38 MAP kinase inhibitor SB203580 ($10 \, \mu m$)

	TPS (ms)	TR$_{90}$ (ms)	$+$ dLdt ($\mu m \, s^{-1}$)	$-$ dLdt ($\mu m \, s^{-1}$)
Control	169 ± 7	244 ± 15	126.6 ± 8.0	-107.5 ± 8.2
MDA 10^{-9} M	153 ± 7	272 ± 16	124.6 ± 10.4	-90.4 ± 8.0
MDA 10^{-8} M	164 ± 9	268 ± 17	113.7 ± 11.3	-91.7 ± 10.6
MDA 10^{-7} M	163 ± 8	255 ± 18	$96.9 \pm 9.2*$	$-75.2 \pm 7.9*$
MDA 10^{-6} M	163 ± 7	268 ± 21	$78.9 \pm 7.7*$	$-61.8 \pm 6.9*$
MDA 10^{-5} M	$148 \pm 5*$	252 ± 20	$67.7 \pm 7.8*$	$-52.3 \pm 8.4*$
MDA 10^{-4} M	$143 \pm 7*$	249 ± 20	$57.7 \pm 6.4*$	$-46.5 \pm 6.2*$
MDA 10^{-3} M	$144 \pm 6*$	248 ± 30	$53.4 \pm 6.5*$	$-53.0 \pm 8.5*$
Control $+$ SB203580	156 ± 7	245 ± 25	139.3 ± 16.6	-115.3 ± 13.2
MDA 10^{-4} M $+$ SB203580	155 ± 7	257 ± 28	132.3 ± 13.9	-129.2 ± 19.9
MDA 10^{-3} M $+$ SB203580	155 ± 6	259 ± 24	122.7 ± 13.9	-106.0 ± 12.7

TPS, Time-to-peak shortening: TR$_{90}$, time-to-90% relengthening: $\pm$ dLdt, maximal velocities of shortening and relengthening. Data represent mean $\pm$ s.e.m., $n = 20$–22 cells, *$P < 0.05$ vs control value.
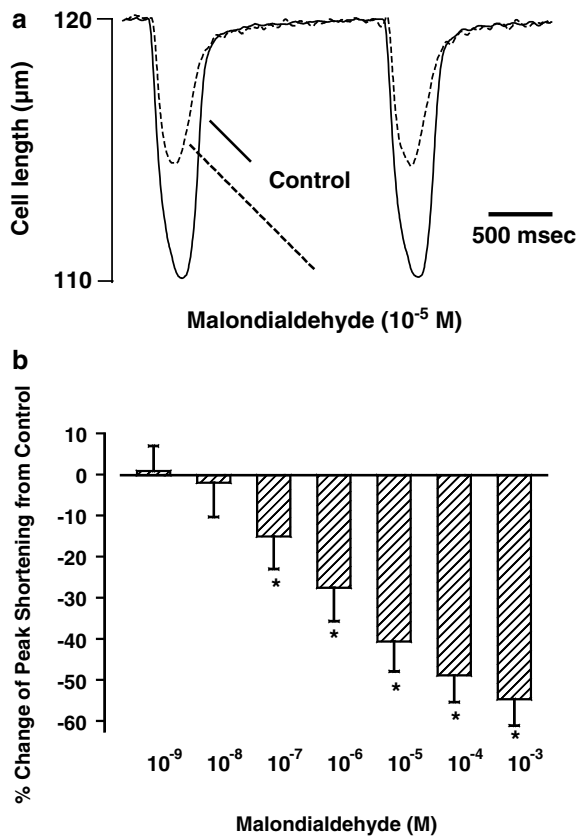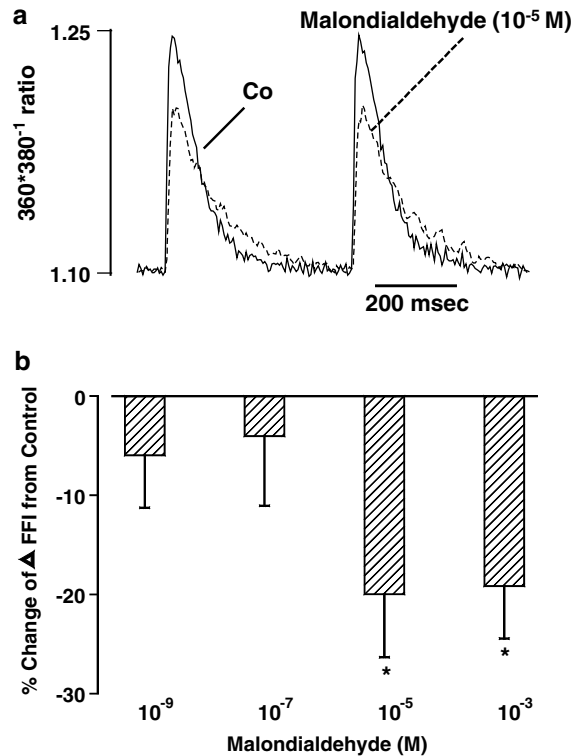
Figure 1 (a) Representative traces depicting the effect of malondialdehyde (10^{-5} M) on cell shortening in ventricular myocytes. (b) Concentration-dependent response of malondialdehyde (10^{-9}–10^{-3} M) on peak cell shortening. Data are presented as percent change from basal PS, which was $9.22 \pm 0.83\%$. Mean $\pm$ s.e.m., $n = 22$ per data group, *$P < 0.05$ vs baseline value.

supported the notion of direct involvement of p38 MAP kinase in malondialdehyde-induced cardiac depression.

Discussion

Our study provided evidence for the first time that the lipid peroxidation end product malondialdehyde directly depresses cardiac contraction and maximal velocity of contraction and relaxation associated with a reduction of intracellular Ca^{2+} transients in isolated ventricular myocytes. The malondialdehyde-induced cardiac depression may involve enhanced oxidative stress and activation of MAP kinase indicated by our pharmacological and immunoblotting studies. In addition, malondialdehyde shortened duration of shortening and had little effect on resting intracellular Ca^{2+} levels and intracellular Ca^{2+} clearing rate. These results may suggest a convincing link between the elevated lipid peroxidation end product malondialdehyde and impaired ventricular contractile function under pathological conditions such as heart failure.

Peroxidation of membrane phospholipids has been suspected to be a major mechanism of oxidant injury and impaired cardiac contractile function (Braughler *et al.*, 1986; Durot *et al.*, 2000). Levels of 25–140 nM malondialdehyde have been reported in human plasma following certain cardiac


Figure 2 (a) Representative traces depicting effect of malondialdehyde (10^{-5} M) on intracellular Ca^{2+} transient changes (ΔFFI) in ventricular myocytes. (b) Concentration-dependent response of malondialdehyde (10^{-9}–10^{-3} M) on ΔFFI. Data are presented as percent change from respective basal ΔFFI value. Mean $\pm$ s.e.m., $n = 29$ per data group, *$P < 0.05$ vs baseline value.
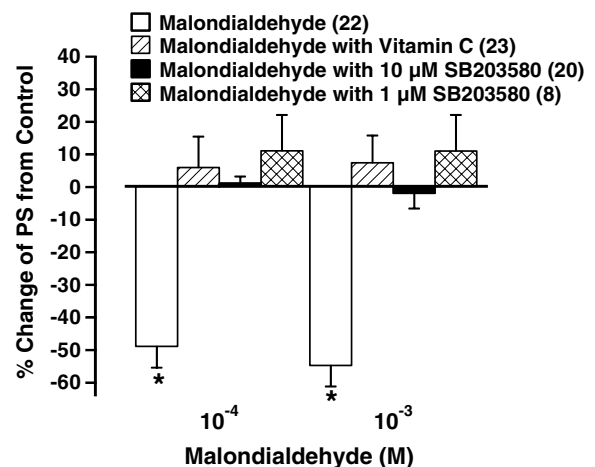
Figure 3 Effect of the antioxidant vitamin C and p38 MAP kinase inhibitor SB203580 on malondialdehyde-induced depression of myocyte shortening. Vitamin C (100 μM) or SB203580 (1 and 10 μM) was applied prior to the addition of malondialdehyde (10^{-4} and 10^{-3} M). Mean $\pm$ s.e.m., $n = 20$–23 per data group, *$P < 0.05$ vs baseline value.

injury such as ischemia–reperfusion (Yeo *et al.*, 1994; Cighetti *et al.*, 1999), consistent with the effective concentration range found in our study. Oxidation of membrane lipid is a process generated by ROS and is responsible for the membrane dysfunction under various disease states (Kaneko *et al.*, 1994; Porter *et al.*, 1995; Dhalla *et al.*, 1996; Buffon *et al.*, 2000). The
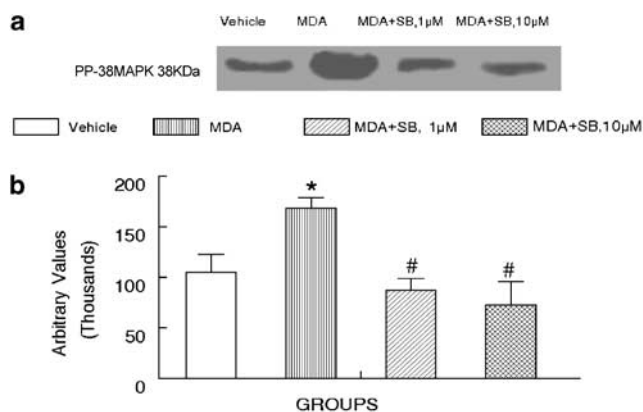
Figure 4 Effect of malondialdehyde (MDA, 10^{-4} M) on phosphorylation of p38 MAP kinase in ventricular myocytes in absence or presence of the p38 MAP kinase inhibitor SB203580 (SB, 1 and 10 μM). (a) Representative gel blot showing p38 MAP kinase phosphorylation (pp-MAPK). (b) Bar graph depicting summary of three different isolations. Mean $\pm$ s.e.m., *$P < 0.05$ vs control (vehicle) group, $P < 0.05$ vs malondialdehyde group.

ROS radicals may initiate a rapid self-propagating chain reaction by attacking the polyunsaturated fatty acids in the membrane. Polyunsaturated fatty acids display the highest sensitivity among cellular macromolecules to ROS-induced damage (Porter *et al.*, 1995; Dhalla *et al.*, 1996). Lower degree of fatty acid unsaturation of cell membrane is believed to be protective against lipid oxidation-derived damage. Membrane damage due to ROS-induced lipid peroxidation has been considered the predominant mechanism for cellular membrane dysfunction and subsequently, alteration of cellular function (Porter *et al.*, 1995; Dhalla *et al.*, 1996). Malondialdehyde is generated through disruption of membrane lipids following lipid peroxidation and is especially dangerous for the viability of cells or tissues. It may be responsible for the compromised ventricular function under conditions where there is elevated lipid peroxidation. It may be speculated that enhanced oxidative stress may trigger the lipid peroxidation-induced ventricular dysfunction. Despite extensive effort in the field of lipid peroxidation, it has not yet been determined if it is a cause or a consequence of several pathological conditions. Results from the current study indicate that malondialdehyde may be a trigger for cardiac dysfunction. The fact that peak cardiac contraction, duration and velocity of contraction and relaxation were altered by malondialdehyde indicated that lipid peroxidation may affect different cardiac contractile or regulatory components.

Although the mechanism(s) of action underlying reduced myocyte contraction in response to malondialdehyde is not fully clear at this time, several speculations may be made. First, the observation that the antioxidant vitamin C abolished the malondialdehyde-induced negative contractile response suggests that malondialdehyde may elicit its inhibitory effect associated with enhanced oxidative stress in the heart. Oxidative stress and damage are known to impair cardiac contractile function (Goldharber & Qayyum, 2000). Mediators or signaling of oxidative stress such as superoxide anion, hydroxyl radical and peroxynitrite have been reported to depress cardiac contractile function directly (Satoh & Matsui, 1997; Ferdinandy *et al.*, 2000). Vitamin C (ascorbate) has been shown to scavenge free radicals, thus preventing

cardiac contractile failure. It is a water-soluble antioxidant and plays an indirect role in terminating lipid peroxidation. It reduces tocopheroxyl radicals thereby elevating the levels of the reduced tocopherol within the tissues (Leung *et al.*, 1981). Tocopherol, on the other hand, is also a lipid-soluble antioxidant with the sole physiological role of directly terminating lipid peroxidation through stabilization of lipid radical by donating a single electron (Burton *et al.*, 1985; Klein *et al.*, 1989). Although direct action of antioxidant such as vitamin C on malondialdehyde-induced membrane damage has not been elucidated, antioxidants have been demonstrated to quench effectively ROS-induced lipid peroxidation and ameliorate certain aspects of biomolecular damage (Dietrich *et al.*, 2002). Secondly, the observation that malondialdehyde may inhibit myocyte shortening at concentrations (such as 10^{-7} M) where no obvious effect on intracellular Ca^{2+} transients was observed indicates that malondialdehyde may alter myofilament Ca^{2+} sensitivity, or other Ca^{2+}-independent machineries.

p38 MAP kinase has been shown to be involved in cellular responses to various assaults such as UV light, osmotic stress, mechanical, oxidative (ROS) and chemical stress (Martin-Blanco, 2000; Obata *et al.*, 2000; Kulisz *et al.*, 2002). Results from our current study provided evidence that p38 MAP kinase activation may mediate malondialdehyde-elicited cardiac contractile inhibition. This is consistent with the finding that malondialdehyde directly facilitate p38 MAP kinase phosphorylation. p38 MAP kinase activation has been shown to elicit a robust negative inotropic effect in intact cardiac myocytes (Liao *et al.*, 2002). As increased p38 MAPK activation is associated with the onset of heart failure (Adams *et al.*, 1998), ischemic or reperfusion injury, and *in vivo* pressure overload (Wang *et al.*, 1998; Ma *et al.*, 1999), the negative inotropic effect of p38 MAPK may contribute, at least in part, to the diminished cardiac contractility under those pathological circumstances. It is speculated that accumulation of ROS may lead to lipid peroxidation and production of malondialdehyde en route to p38 MAP kinase activation and cardiac depression. Indeed, a number of studies have demonstrated that inhibiting p38 MAP kinase signaling improves contractile function in ischemia/reperfusion-injured hearts (Cain *et al.*, 1999; Ma *et al.*, 1999). This notion not only reveals an unappreciated function of p38 MAP kinase but also demonstrates a novel mechanism for stress-activated signals in the regulation of cardiac contractility. Interestingly, the negative inotropic effect of p38 MAP kinase is believed to be mediated by reducing the responsiveness of myofilaments to Ca^{2+} (Liao *et al.*, 2002), consistent with our findings in the discrepant threshold of malondialdehyde-induced inhibition between myocyte shortening and intracellular Ca^{2+} transient. Another interesting observation from our current study is that SB203580 inhibits the dual phosphorylation of p38 MAP kinase. These data may suggest that the malondialdehyde-induced activation of p38 MAP kinase could possibly arise from an autophosphorylation reaction of p38 MAP kinase. Autophosphorylation has been indicated to be an alternative activation pathway contributing to the biological response of p38 MAP kinase (Ge *et al.*, 2002). Nevertheless, with the limited information from this study we can only speculate but cannot suggest such autophosphorylation mechanisms involved in malondialdehyde-induced cardiac response.

In conclusion, our study demonstrates direct cardiac depression by malondialdehyde at the ventricular myocyte level, possibly through oxidative stress. These findings not only reveal a novel function for p38 MAPK but also provide new insights for better understanding of enhanced p38 MAPK signaling in cardiac dysfunction under certain pathophysiological conditions, such as cardiac ischemic/reperfusion injury or chronic heart failure. The precise nature of the cardiac contractile effects of malondialdehyde is still not clear. Future studies should focus on its action on cardiac excitation–contraction coupling and membrane ion channels. These approaches will be essential to further our understanding of the cellular effects, pharmacological profiles as well as clinical applications of malondialdehyde and lipid peroxidation.

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