Neutrophil-mediated accumulation of 2-ClHDA during myocardial infarction: 2-ClHDA-mediated myocardial injury

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Thukkani, Arun K., Bradley D. Martinson, Carolyn J. Albert, George A. Vogler, and David A. Ford. Neutrophil-mediated accumulation of 2-ClHDA during myocardial infarction: 2-ClHDA-mediated myocardial injury. Am J Physiol Heart Circ Physiol 288: H2955–H2964, 2005. First published January 28, 2005; doi:10.1152/ajpheart.00834.2004.—The pathophysiological sequelae of myocardial infarction include neutrophil infiltration into the infarct zone that contributes to additional damage to viable tissue and removal of cellular debris from necrosed tissue. Reactive chlorinating species produced by myeloperoxidase amplify the oxidant capacity of activated neutrophils. Plasmalogens are a major phospholipid subclass of both endothelial cells and cardiac myocytes. Recent studies have shown that plasmalogens are targeted by neutrophil-derived reactive chlorinating species that lead to the production of α-chloro fatty aldehydes. Results herein demonstrate that the α-chloro fatty aldehyde 2-chlorohexadecanal (2-ClHDA) accumulates in rat hearts subjected to left anterior descending coronary artery occlusion. Myocardia from rats subjected to surgical infarction had increased 2-ClHDA and neutrophil infiltration levels compared with myocardia from rats subjected to sham surgery. Additionally, infarcted myocardia from rats rendered neutropenic by treatments with an anti-neutrophil antibody had diminished 2-ClHDA and neutrophil infiltration levels compared with infarcted myocardia from normopenic rats; 2-ClHDA was shown to elicit myocardial damage as determined by lactate dehydrogenase release in isolated perfused rat hearts. Additionally, 2-ClHDA treatment of hearts resulted in decreased heart rate and ventricular performance. Taken together, the present results demonstrate a novel neutrophil-dependent myeloperoxidase-based mechanism that results in 2-ClHDA production in response to regional myocardial infarction that may also contribute to cardiac dysfunction.

2-chlorohexadecanal; myeloperoxidase; ischemia; fatty aldehyde

2-ClHDA has been shown (40) to be a potent chemoattractant as in human atherosclerotic lesions (39–41). In addition, 2-ClHDA has been shown to disrupt erythrocyte membrane dynamics (17, 33, 34, 44). However, it should be noted that lipid chlorohydrins have not been detected in vivo. Furthermore, until recently, lipid products derived from RCS attack have not been demonstrated in vivo (41).

Plasmalogens are a glycerophospholipid molecular subclass that possesses a vinyl ether bond linking the sn-1 aliphatic group to the glycerol backbone. Plasmalogens are also a major phospholipid found in cells of the cardiovascular system including cardiac myocytes, endothelial cells, and vascular smooth muscle (12, 14, 26). Biological roles of plasmalogens likely include serving as storage depots for esterified arachidonic acid, as boundary lipids that solvate transmembrane proteins, and as a nonpropagating target that terminates free-radical peroxidation reactions (12, 13, 46, 47). Recently, the vinyl ether bond of plasmalogens has been shown (1) to be targeted for oxidation by MPO-derived RCS to result in the production of α-chloro fatty aldehydes including 2-chlorohexadecanal (2-ClHDA). We have demonstrated the in vivo accumulation of the RCS-derived lipid product α-chloro fatty aldehyde in both activated neutrophils and monocytes as well as in human atherosclerotic lesions (39–41). In addition, 2-ClHDA has been shown (40) to be a potent chemoattractant in vitro that may recruit circulating neutrophils to areas of inflammation. Because neutrophils infiltrate infarcted myocardium, the present study was designed to determine whether neutrophil-derived RCS attack myocardial plasmalogens in infarcted myocardium. The present results demonstrate that in...
a rat model of myocardial infarction, myocardial 2-ClHDA levels are elevated compared with myocardia from rats subjected to sham surgery. It is likely that 2-ClHDA in infarct zones is mediated by the neutrophil infiltrate, because this halogenated fatty aldehyde did not accumulate in infarct zones of rats rendered neutropenic. Myocardial injury was also elicited by 2-ClHDA in studies on Langendorff-perfused rat hearts. Taken together, the results herein demonstrate for the first time the production of 2-chloro fatty aldehyde in infarcted myocardium and suggest that it may contribute to further myocardial cell death.

MATERIALS AND METHODS

Materials. We synthesized and purified 2-ClHDA and 2-Cl-[d13]-HDA as previously described (40). Rabbit anti-rat neutrophil antibody was purchased from Accurate Chemicals. Pentafluorobenzyl (PFB) hydroxylamine was purchased from Aldrich.

In vivo chronic left anterior descending coronary artery ligation in rats. All animal procedures were conducted in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, Washington, DC, 1996) and were approved by the Animal Care Committee of Saint Louis University. Chronic ligation of the left anterior descending (LAD) coronary artery was performed as previously described (11). In brief, male Sprague-Dawley rats (250–300 g body wt) were injected with ketamine-xylazine (55 and 7 mg/ml, respectively; 0.1 ml/100 g ip). After induction of anesthesia, a tail-vein catheter was secured, rats were orotracheally intubated and injected with buprenorphine (0.05 mg/ml; 0.1 ml/100 g ip), the left thoracic area was clipped, and surgical scrub was applied. Animals were then mechanically ventilated with 100% O2 (Harvard Apparatus). A left lateral thoracotomy was performed. The thoracic cage was exposed, and the intercostal space between ribs 4 and 5 was separated with a retractor. The pericardium was lifted away from the heart and a pericardiotomy was performed. The left atrial appendage was retracted, and a 6-0 suture was placed around the proximal LAD by passing the suture from under the right edge of the left atrial appendage through to the left edge of the pulmonary cone. This LAD suture was tied tightly or loosely to produce an infarction or sham surgery, respectively, the thoracic incision was closed with 5-0 suture, and meipivacaine was applied to the incision area. After recovery from surgery, rats were weighed and individually housed. At selected postsurgical intervals, 100 µl of venous blood was collected, and subsequently, hearts were removed and frozen in liquid N2 until analysis. For histology, excised hearts were immediately fixed in formalin for sectioning and hematoxylin-eosin staining. Histological images were captured with a Nikon 990 Coolpix camera. For triphenyltetrazolium staining, hearts were cut into cross-sectional slices and subsequently stained using the method described by Downey and coworkers (45).

As indicated, rats were rendered neutropenic by injection of rabbit anti-rat neutrophil antibody (1 ml ip; 1.5 dilution in saline) 1 day before LAD occlusion (36). Immediately before surgery as well as after experimental intervals, 100 µl of venous blood was collected, differentially stained, and visually examined to confirm neutropenia. This dosing regimen resulted in a complete absence of circulating neutrophils that lasted for ~3 days.

Myocardial lipid extraction and TLC purification of 2-ClHDA. Frozen whole hearts from rats subjected to either LAD ligation or sham surgery were pulverized at the temperature of liquid N2. The lipids from pulverized myocardial tissue (~100 mg) were sequentially extracted by the method of Bligh and Dyer (5) in the presence of the internal standard, 2-Cl-[d13]-HDA. The α-chloro fatty aldehydes contained in the crude lipid extracts were then purified by TLC using silica gel G plates as the stationary phase and a 90:10:1 (vol/vol/vol) petroleum ether-ethyl ether-acetic acid solution as the mobile phase. Silica corresponding to regions of the TLC plate (Whatman) that 2-ClHDA migrated to (relative migration, 0.46) was scraped and extracted into chloroform (1, 5). The lipid-containing chloroform extracts were evaporated under N2, and reaction products were converted to their respective PFB oxime derivatives and analyzed by gas chromatography-mass spectrometry (GC-MS) as described below. Additionally, myocardial phospholipid-derived inorganic phosphate content was determined from crude lipid extracts (7).

Preparation of PFB oximes of α-chloro fatty aldehydes and GC-MS analysis. TLC-purified 2-ClHDA from myocardial lipid extracts was converted to its respective PFB oxime derivative before analysis by GC-MS as previously described (40). In brief, the dried lipid extracts were resuspended in 300 µl of ethanol and treated with 300 µl of 6 mg/ml PFB hydroxylamine for 25 min at 24°C, and the derivatization was terminated by addition of 1.2 ml of H2O. Reaction products were then sequentially extracted, concentrated, and injected onto the GC column. GC-MS analysis of PFB oximes of α-chloro fatty aldehydes was performed on a Hewlett-Packard (Palo Alto, CA) 5973 N gas chromatograph coupled to a Hewlett-Packard 6890 gas chromatograph using the negative-ion chemical ionization (NICI) mode with methane as the reagent gas. The source temperature was set at 150°C. The electron energy was 70 eV, and the emission current was 300 µA. The PFB derivatives were separated on a DB-1 column (12 m length, 0.2 mm inner diameter, and 0.33 µm film thickness; J&W Scientific; Folsom, CA). The inlet and the transfer-line temperatures were maintained at 250 and 280°C, respectively. The GC oven was maintained at 150°C for 3.5 min, increased at a rate of 25°C/min to 310°C, and held at 310°C for an additional 5 min. Quantitation of 2-ClHDA was performed using selected ion monitoring (SIM) GC-MS as previously described (40). Specifically, the total integrated peak area arising from the mass-to-charge ratio (m/z) of 288, the structurally informative fragment produced from the PFB oxime of 2-ClHDA, was compared with the total integrated peak area produced by the m/z of 292, the structurally informative fragment ion of the PFB oxime of 2-Cl-[d13]-HDA. Myocardial 2-ClHDA levels were normalized to myocardial phospholipid-derived inorganic phosphate.

Myocardial MPO activity measurements. MPO measurements of surgically treated myocardia were performed as previously described (6). In brief, pulverized tissue (~50 mg) was suspended in 1 ml of 0.5% hexadecyltrimethylammonium bromide in 50 mM potassium phosphate buffer, pH 6, and homogenized. Samples were then sonicated for 10 s and freeze-thawed with liquid N2 three times. Suspensions were centrifuged at 45,000 g for 45 min at 4°C. Of the resulting supernatant, 100 µl was mixed with 2.9 ml of 50 mM phosphate buffer, pH 6, that contained 0.167 mg/ml o-diisindoline dihydrochloride and 0.0005% H2O2. The change in absorbance at 460 nm and 25°C was monitored spectrophotometrically (Beckman). One unit of MPO activity was monitored as that by degrading one micromol H2O2 in 1 min at 25°C. Myocardial MPO activity was normalized to myocardial phospholipid-derived inorganic phosphate.

Treatment of Langendorff-perfused rat hearts with 2-ClHDA. Langendorff-perfused rat hearts were prepared as previously described (27). In brief, male Sprague-Dawley rats (250–300 g body wt) were injected with heparin (800 U/kg ip) 10 min before they were anesthetized with pentobarbital sodium (125 mg/kg ip). After induction of anesthesia, the hearts were excised and placed in ice-cold saline before being perfused. Hearts were retrograde perfused via the aorta (Langendorff perfused) with modified Krebs-Henseleit buffer (KHB) that consisted of (in mM) 118 NaCl, 4.7 KCl, 3 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 0.5 Na-EDTA, 15 NaHCO3, and 11 mM glucose and was equilibrated with 95% O2-5% CO2, pH 7.4 at a constant aortic perfusion pressure of 80 mmHg at 37°C.

The 2-ClHDA was prepared as a 10× concentration in KHB supplemented with 0.1% ethanol immediately before use for perfusions. The 10× 2-ClHDA buffer was injected via a sidearm cannula over 1 min at a constant flow rate that equaled one-tenth of the

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coronary flow rate to provide a 1× concentration of 2-ClHDA while maintaining the coronary flow rate (typically 12–15 ml/min). After delivery of the 2-ClHDA via the sidearm cannula, the cannula was flushed via a 1-min infusion of KHB. Control treatments comprised an injection of KHB supplemented with 0.1% ethanol and a subsequent 1-min KHB infusion through the sidearm cannula. To monitor myocardial function, a 6-F pressure-transducer catheter (Millar) was inserted into the left ventricle. Left ventricular pressure, dP/dt, and heart rate were then continuously recorded using Macintosh PowerLab hardware and software. Specifically, 10-s intervals at the indicated time points were analyzed for average maximum left ventricular pressure (LVPmax), first derivative of left ventricular developed pressure (dP/dtmax), and average heart rate. Additionally, cardiac effluent was collected over 1 min at the indicated time points to determine coronary blood flow rate and LDH release (measured using an LDH assay kit from Sigma). After the 2-h perfusion interval, hearts were blotted dry, weighed, and immediately frozen in liquid N2. Hearts were then pulverized under liquid N2, and a portion of the resulting tissue homogenate was used to determine the dry weight. Coronary flow rate and total heart dry weight were both used to normalize LDH release into cardiac effluent.

**Statistical analysis.** Statistical analysis was performed between multiple groups using ANOVA and the post hoc Newman-Keuls test. Correlation was determined by linear regression analysis.

**RESULTS**

To determine that 2-ClHDA is produced in rat hearts subjected to 1 day of LAD occlusion, 2-ClHDA was sequentially purified from myocardial lipid by TLC purification of 2-ClHDA and was subsequently converted to its PFB oxime derivative. The mass spectrum of this product using NICI (Fig. 1A) had an identical GC retention time (~8.4 min) and fragmentation pattern compared with the PFB oxime of authentic 2-ClHDA (Fig. 1B). The ion pair m/z, which were 35/37 in both spectra, were present at a 3:1 abundance ratio due to the natural abundances of 35Cl and 37Cl. Similarly, the fragment ions m/z of 288 and 290 were present in a 3:1 ratio of abundance that is characteristic of monochlorinated molecules, and these two fragment ions are structurally informative fragment ions of the PFB oxime of 2-ClHDA because they are
fragmented from the 2-ClHDA-derived portion of the derivative (Fig. 1A, inset). Other fragment ions had a m/z of 414 [parent ion (M⁻)-HF-Cl] as well as 178 and 196, which are fragment ions of the PFB group (Fig. 1A, inset; Ref. 20). Collectively, these data indicate that 2-ClHDA is present in myocardia of rats subjected to LAD occlusion for 1 day.

This GC-MS technique (NICI of the PFB oxime of 2-ClHDA) was exploited to quantitate myocardial 2-ClHDA in hearts subjected to LAD occlusion. The synthetic internal standard 2-Cl-[d₄]-HDA was prepared, and quantification of myocardial 2-ClHDA was achieved by comparing the peak areas of the structurally relevant fragments at a m/z of 288 to that of the internal standard, which had a m/z of 292, using SIM of these ions. SIM for the m/z of 288 from PFB derivatives from rat hearts subjected to 1 day of LAD occlusion resulted in two peaks (Fig. 2A). These peaks had identical retention times to the peaks corresponding to a m/z of 288 from the PFB oxime of authentic 2-ClHDA (i.e., ~8.3 and 8.4 min) and correspond to the syn and anti isomers characteristic of PFB oxime derivatives (20). Concurrent SIM for the m/z of 292, which is the structurally informative fragment ion derived from the PFB oxime of the internal standard 2-Cl-[d₄]-HDA, also resulted in two peaks (Fig. 2A). In contrast, SIM analysis of PFB derivatives from rat hearts subjected to either sham surgery (and harvested 1 day after the surgery; Fig. 2B) or LAD occlusion followed by immediate harvest of heart tissue (zero time LAD occlusion; C). Triphenyltetrazolium-stained hearts from rats subjected to 1-day LAD occlusion (D) or 1-day sham surgery (E) are shown. Cleared (white) area of the infarct zone is indicated (arrow).

**Fig. 2.** Selected ion monitoring (SIM) GC-MS analysis of myocardial 2-ClHDA. Animals were subjected to either chronic LAD occlusion for 1 day or sham surgery followed by 1 day of recovery from surgery. After experimental intervals, myocardial lipids were prepared for 2-ClHDA analysis by SIM GC-MS. SIM GC-MS analysis using NICI for mass-charge ratios (m/z) of 288 and 292 derived from 2-ClHDA prepared from a rat subjected to either 1 day of LAD occlusion (A), sham surgery followed by 1 day of recovery from surgery (B), or LAD occlusion followed by immediate harvest of heart tissue (zero time LAD occlusion; C). Triphenyltetrazolium-stained hearts from rats subjected to 1-day LAD occlusion (D) or 1-day sham surgery (E) are shown. Cleared (white) area of the infarct zone is indicated (arrow).

**Fig. 3.** Levels of 2-ClHDA in infarcted myocardia. Rats were subjected to either chronic LAD occlusion or sham surgery. Alternatively, some rats were rendered neutropenic before LAD occlusion. At indicated experimental intervals, myocardial lipids were prepared for 2-ClHDA analysis by SIM GC-MS. Values are means ± SE; *P < 0.01; **P < 0.005; ***P < 0.001.
ing of rat hearts subjected to LAD occlusion for 1 day as well as those subjected to sham surgery and harvested 1 day later confirmed that the hearts subjected to LAD occlusion had significant infarct areas, whereas hearts subjected to sham surgery had no infarct zones (Fig. 2, D and E).

Figure 3 summarizes data for the quantification of myocardial 2-ClHDA content by SIM-GC-MS analysis. Approximately 4.5 and 2.75 pmol 2-ClHDA/μmol phospholipid phosphate accumulated in 1- and 2-day infarcted myocardia, respectively (Fig. 3). There was also a modest increase in 2-ClHDA content in hearts from rats that were subjected to sham surgery with either a 1- or 2-day recovery from sham surgery compared with the 2-ClHDA content in hearts that were harvested from animals immediately after either sham surgery or LAD occlusion. The increase in 2-ClHDA content in the sham-surgery hearts coincides with an increase in MPO activity in these hearts (Fig. 4) as well as neutrophil and MPO staining at the epicardial surface of these hearts (Figs. 5 and 6). The presence of 2-ClHDA and epicardial neutrophils in the sham-surgery-treated rats is likely due to an inflammatory response to the pericardiotomy performed during surgery (35).

The decrease in myocardial 2-ClHDA content between 1 and 2 days of LAD occlusion may be due to catabolism of 2-ClHDA and/or a decrease in active neutrophils over this time period. This latter possibility is suggested by decreased myocardial MPO-activity measurements from hearts subjected to 2 days of LAD occlusion compared with measurements from hearts subjected to 1 day of LAD occlusion (see Fig. 4) as well as the fewer neutrophils that were present in hearts subjected to 2 days of LAD occlusion (see Figs. 5 and 6). In contrast, rendering animals neutropenic before LAD occlusion severely decreased myocardial 2-ClHDA content in rats subjected to 1 and 2 days of LAD occlusion (~0.24 and ~0.16 pmol 2-ClHDA/μmol phospholipid phosphate, respectively; see Fig. 3). Figure 7 shows the efficiency of antibody treatment to elicit neutropenia within the time frame of the LAD occlusion protocols used in these studies (i.e., neutropenia was maintained over a 3-day interval). Myocardial MPO activities in

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**Fig. 4.** Myeloperoxidase (MPO) activity in myocardia. Hearts from animals subjected to either LAD occlusion, sham surgery, or LAD occlusion after induction of neutropenia were harvested at the indicated time points and analyzed for cardiac MPO activity. Values are means ± SE; *P < 0.01; **P < 0.005; ***P < 0.001.

**Fig. 5.** Hematoxylin-eosin staining of rat myocardia. Rats were subjected to either LAD occlusion or sham surgery. At 0, 1, or 2 days after surgery, animals were killed and hearts were harvested and immediately fixed in formalin in preparation for hematoxylin-eosin staining. Images (magnification, ×40) were captured from tissue subjected to 0-day LAD occlusion (LAD occlusion with immediate harvest of the heart; A), 1-day LAD occlusion (B), 2-day LAD occlusion (C), 0-day sham surgery (D), 1-day sham surgery (E), 2-day sham surgery (F), 0-day neutropenia with LAD occlusion (G), 1-day neutropenia with LAD occlusion (H), and 2-day neutropenia with LAD occlusion (I). Presence of neutrophils on the epicardial surface is indicated (arrows).
hearts from neutropenic rats subjected to LAD occlusion for 1 and 2 days were reduced (≈0.55 and ≈0.033 U of MPO activity/μmol phospholipid, respectively; see Fig. 4). Additionally, in contrast with myocardia from normal rats subjected to LAD occlusion, as confirmed by histological analysis, there was an absence of neutrophils in myocardia from neutropenic rats subjected to LAD occlusion (see Figs. 5 and 6). Figure 8 shows the correlation between myocardial neutrophil content (myocardial MPO) and myocardial 2-ClHDA level for individual hearts subjected to the conditions shown in Figs. 3 and 4. Collectively, the increased presence of activated neutrophils in infarcted myocardia likely leads to greater targeting of plasmalogens by MPO-derived RCS and therefore increased myocardial 2-ClHDA content. Furthermore, results from neutropenic animals emphasize that a source of MPO (i.e., neutrophils) is strictly required for 2-ClHDA production in response to myocardial infarction.

Next, Langendorff-perfused rat hearts were treated with 2-ClHDA, and cardiac function was monitored. Data shown in Fig. 9 demonstrate that compared with control perfused hearts, both the average dP/dtmax (Fig. 9A) and the average LVPmax (Fig. 9C) values were significantly decreased at 30-, 60-, 90-, and 120-min time points after a 1-min perfusion with 20 μM 2-ClHDA. Decreases in heart rate were also observed after treatments with 20 μM 2-ClHDA (Fig. 9B). Also, 20 μM 2-ClHDA elicited decreases in coronary flow rate in isolated perfused hearts (Fig. 9D). In contrast, 1-min treatments with 2 μM 2-ClHDA did not elicit significant changes in dP/dtmax and LVPmax, coronary flow rate, and heart rate (Fig. 9). Additionally, as shown in Fig. 10, the LDH content in coronary effluents from hearts treated for 1 min with 20 μM 2-ClHDA was significantly increased at both 30 and 60 min after treatments compared with control perfused hearts at these time points. In contrast, treatments with 2 μM 2-ClHDA did not elicit LDH release into coronary effluents (Fig. 10). An intermediate response was observed in hearts that were treated for 1 min with 10 μM 2-ClHDA with significant elevations in LDH content in the coronary effluent observed 30 min after treatments (Fig. 10). Taken together, the ex vivo results presented here collectively suggest that 20 μM 2-ClHDA initially
inflicts substantial myocardial injury and subsequently results in decreased cardiac function in Langendorff-perfused rat hearts compared with either control or 2 μM 2-ClHDA.

DISCUSSION

Recently, MPO-derived RCS from activated neutrophils have been shown to oxidize the sn-1 vinyl ether bond of plasmalogens to result in the production of the α-chloro fatty aldehyde 2-ClHDA (40). However, before the findings described herein, the targeting of myocardial phospholipids by RCS had not been demonstrated. In the present study, NICI GC-MS analysis of TLC-purified myocardial lipid extracts was used to identify 2-ClHDA in myocardia subjected to chronic LAD occlusion leading to infarction. In infarcted myocardia, 2-ClHDA was identified by demonstrating that the PFB oxime of myocardial 2-ClHDA has an identical retention time when subjected to GC with the PFB oxime of authentic 2-ClHDA. Furthermore, the PFB oxime of myocardial 2-ClHDA has a nearly identical mass spectrum to that of the PFB oxime of authentic 2-ClHDA when subjected to NICI-GC-MS. This represents the first time that RCS-derived lipid products have been observed in myocardium. Furthermore, it is likely that 2-ClHDA accumulation in hearts subjected to myocardial infarction is likely mediated by RCS attack of plasmalogens, since our previous studies have shown that the masked aldehyde vinyl ether of plasmalogens is targeted by RCS and results in the release of α-chloro fatty aldehydes (1).

Several investigations have suggested that RCS may have a role in ischemic reperfusion or infarcted myocardia (3, 15, 28, 43). Askari et al. (3) demonstrated that RCS released from neutrophils chlorinate and inactivate myocardial plasminogen activator inhibitor-1. Others have demonstrated that the RCS HOCl itself mediates several cardiotoxic effects including the modification of cardiac β-adrenoceptors and adenylyl cyclase (31), inhibition of cardiac Na⁺-K⁺-ATPase activity (23), perturbation of Ca²⁺ homeostasis (10), as well as increase of the number of reperfusion-induced arrhythmias (28). Because we have previously shown that 2-ClHDA is produced from RCS attack of plasmalogens (1, 40), it is possible that at least some of the effects of RCS on the myocardium could be mediated by the loss of sarcolemmal plasmalogens as well as the production of the reactive halogenated fatty aldehyde 2-ClHDA.

The present results show that 4.5 pmol 2-ClHDA per 1 μmol phospholipid phosphate is present in 1-day-infarcted myocardium. Assuming that 2-ClHDA is evenly distributed throughout cellular water and is localized to the infarct zone, the estimated concentration of 2-ClHDA produced in response to infarction is ~1 μM. It is likely, however, that this is an underestimation of the 2-ClHDA concentration produced at the site of inflammation in infarcted myocardium. First, fatty aldehydes readily form Schiff base adducts with primary amine groups of both proteins and the phosphatidylethanolamine head group, which would reduce free 2-ClHDA levels over time (21). Second, infarct levels of 2-ClHDA may be further reduced over time by washout into blood via collateral vessels as well as by sequential oxidation to a fatty acid and subsequent β-oxidation (32). Third, previous studies suggest that the concentration of 2-ClHDA produced by activated neutrophils is as much as 260 μM (40). Taken together, it is estimated that the 2-ClHDA concentration in infarcted myocardial regions likely ranges within an order of magnitude of 20 μM. It should be appreciated that treating hearts with either 10 or 20 μM 2-ClHDA elicits cardiac injury as determined by the release of LDH. Additionally, injury elicited by 2-ClHDA treatment led to a progressive decrease after treatment in heart rate, LVP max, dP/dmax, and coronary flow rate. It should also be appreciated that the effects elicited by either 10 or 20 μM 2-ClHDA were after an acute 1-min treatment with 2-ClHDA. Taken together, these ex vivo data suggest that 2-ClHDA...
directly injures myocardial tissue and contributes to the subsequent observed deterioration in cardiac function.

Although the precise biochemical mechanism that mediates damage and/or dysfunction elicited by 2-ClHDA has yet to be determined, it is likely that Schiff base adducts of myocardial proteins and phospholipids with 2-ClHDA are formed in infarcted myocardium. Indeed, Schiff base adducts of proteins with 4-hydroxynonenal, an aldehyde lipid peroxidation product, have been detected in previously ischemic reperfused myocardium (9). Thus one possible mechanism that 2-ClHDA may mediate injury to heart would be the formation of 2-ClHDA-protein adducts that would potentially alter protein function. Because it is likely that 2-ClHDA is produced at the plasma membrane of cells attacked by RCS, it is possible that the localized production of 2-ClHDA at the sarcolemma may result in modification of critical sarcolemmal proteins such as ion channels. It should also be appreciated that because 2-ClHDA is a neutrophil chemoattractant (40), this aldehyde may be important for recruitment of additional neutrophils to infarcted myocardium. Although the precise biological role of 2-ClHDA remains to be elucidated, the generation of 2-ClHDA in infarcted myocardium and the effects of 2-ClHDA on myocardial function suggest that production of 2-ClHDA may represent an important biochemical mechanism that leads to impaired cardiac function in infarcted myocardium.

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REFERENCES


44. Winterbourn CC, van den Berg JJ, Roitman E, and Kuypers FA. Chlorohydrin formation from unsaturated fatty acids reacted with hypo-
D, Cohen MV, and Downey JM. Rat and rabbit heart infarction: effects 
of anesthesia, perfusate, risk zone, and method of infarct sizing. Am J 
46. Zoeller RA, Grazia TJ, LaCamera P, Park J, Gaposchkin DP, and 
Farber HW. Increasing plasmalogens levels protect human endothelial 
cells during hypoxia. Am J Physiol Heart Circ Physiol 283: H671–H679, 
2002.
47. Zoeller RA, Morand OH, and Raetz CR. A possible role for plasmalo-
gens in protecting animal cells against photosensitized killing. J Biol 