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High-performance liquid chromatographic method for the simultaneous detection of malonaldehyde, acetaldehyde, formaldehyde, acetone and propionaldehyde to monitor the oxidative stress in heart

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ABSTRACT

Lipid peroxidation (LPO) is the oxidative deterioration of polyunsaturated fatty acids (PUFA) with the production of lipid hydroperoxides, cyclic peroxides, cyclic endoperoxides, and finally fragmentation to ketones and aldehydes (including malonaldehyde, MDA). Estimation of LPO through MDA formation measured by assaying thiobarbituric acid (TBA) reactive products remains the method of choice to study the development of oxidative stress in tissues. However, MDA estimation by TBA reactive products is non-specific and often gives erroneous results. In this report we describe a method using high-performance liquid chromatographic separation to estimate MDA, formaldehyde (FDA), acetaldehyde (ADA), acetone, and propionaldehyde (PDA), the degradation products of oxygen-derived free radicals (ODFR) and PUFA, as presumptive markers for LPO. Oxidative stress was induced in the tissue by perfusing an isolated rat heart with hydroxyl radical generating system (xanthine + xanthine oxidase + $FeCl_3$ + EDTA). The coronary effluents were collected, derivatized with 2,4-dinitrophenylhydrazine (DNPH), and extracted with pentane. Aliquots of 25 μl in acetonitrile were injected onto a Beckman Ultrasphere C_{18} (3 μm) column. The products were eluted isocratically with a mobile phase containing acetonitrile–water–acetic acid (40:60:0.1, v/v/v), measured at three different wavelengths (307, 325 and 356 nm) using a Waters M-490 multichannel UV detector and collected for gas chromatography–mass spectrometry (GC–MS) analysis. The peaks were identified by co-chromatography with DNPH derivatives of authentic standards, peak addition, UV pattern of absorption at the three wavelengths, and by GC–MS. The retention items of MDA, FDA, ADA, acetone, and PDA were 5.3, 6.6, 10.3, 16.5, and 20.5 min, respectively. The results of our study indicated progressive increase of all five lipid metabolites as a function of the duration of ODFR perfusion. Hydroxyl radical scavengers, superoxide dismutase plus catalase, completely inhibited the formation of these lipid metabolites, demonstrating that the release of lipid metabolites from the isolated heart was indeed in response to oxidative stress. Since MDA, FDA, ADA, acetone, and PDA are the products of ODFR–PUFA interactions, this method allows proper estimation of LPO which monitors the oxidative stress developed during the reperfusion of ischemic myocardium.

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INTRODUCTION

Development of oxidative stress resulting from the generation of oxygen free radicals has been implicated in the pathogenesis of a variety of diseases including atherosclerosis, heart attack, stroke, shock lung, trauma, aging, malaria, and influenza [1,2]. These reactive oxygen species also play an important role in health hazards caused by industrial pollutants, environmental carcinogens, pesticides, cigarette smoke, and deterioration of foods. Oxidative stress can be detected either directly by estimating the presence of free radicals using electron spin resonance spectroscopy (ESR) or high-performance liquid chromatography (HPLC) or indirectly by monitoring the formation of the extent of lipid peroxidation and/or conjugated diene formation. Direct detection of free radicals is a difficult task, firstly, because of the extremely short lives of the free radicals (usually in the range of nanoseconds), and secondly, because of the sophistication in the methodologies. In addition, ESR is a very expensive instrument and not readily available in many laboratories. The measurement of lipid peroxidation, on the other hand, is extremely simple and therefore, the most popular method for the detection of oxidative stress.

Determination of malonaldehyde (MDA) is the most widely recognized method for the monitoring of lipid peroxidation in health related diseases [3]. Several methods are available for the detection of MDA. The most commonly used method consists of the measurement of thiobarbituric acid reactive products formed from the reaction of MDA and thiobarbituric acid (TBA) using spectrophotometric assay method [4,5]. Although this method is relatively simple, the significance of the results is often blunted because of the incorrect interpretation of the results. This is because of the non-specificity of the TBA reactions, TBA not only forms a colored complex with MDA, but it also reacts with many other compounds including ribose, biliverdin, amino pyrimidines, and sialic acid, which may also be present in biological samples [6]. This method was subsequently modified to quantitate the specific MDA–TBA adducts, but the method is not widely used because of the lengthy as well as extremely complex nature of sample preparation [7,8].

Recently we have described a rapid and simple technique for assaying MDA formed during the reperfusion of ischemic myocardium [9]. We now report that this technique can also be used to monitor the development of oxidative stress. Isolated rat heart was perfused with the free radical generating system to generate the reactive oxygen species. The extent of lipid peroxidation was measured by monitoring the formation of MDA and other lipid metabolites after derivatizing with 2,4-dinitrophenylhydrazine (DNPH) using HPLC. The identity of the reaction products obtained by HPLC of coronary perfusates was confirmed for the first time by gas chromatography–mass spectroscopy (GC–MS).

EXPERIMENTAL

Materials

Xanthine, xanthine oxidase, EDTA, TBA and DNPH were obtained from Sigma (St. Louis, MO, USA). Malonaldehyde-bis-dimethyl acetate was purchased from Aldrich (Milwaukee, WI, USA), while formaldehyde (FDA), acetaldehyde (ADA), acetone, and propionaldehyde (PDA) were from Sigma. High purity DNPH standards were prepared by stoichiometric derivatization and repeated crystalization from methanol.

All organic solvents were of HPLC grade (Burdick & Jackson, Muskegon, MI, USA). Water was purified with a Milli-Q system (Millipore, Marlborough, MA, USA). The mobile phase was filtered through a 0.22-μm Nylon-66 solvent filter (Rainin Instrument, Woburn, MA, USA). All other chemicals were of analytical grade.

Methods

Isolated rat heart preparation. Sprague Dawley male rats of about 250 g body weight were anesthetized with intraperitoneal pentobarbital (80 mg per kg). Hearts were removed and quickly mounted on a non-circulating Langendorff perfusion apparatus as described previously [10]. Retrograde perfusion was established at a pressure of 100 cmH_2O (1 $cmH_2O = 98.07$ Pa)

with oxygenated normothermic Krebs–Henseleit bicarbonate (KHB) buffer. Hearts were allowed to be equilibrated for 10 min at 37°C with non-circulating KHB buffer. The heart was then perfused for 45 min with the hydroxyl radical ($OH^{\cdot}$) generating system [xanthine (100 μM), XO (8 mU/ml), $FeCl_3$ (10 μM) and EDTA (100 μM) in the presence or absence of $OH^{\cdot}$ radical scavengers, superoxide dismutase (SOD) (50 U/ml) and catalase (50 U/ml)]. Perfusates were withdrawn before and during the perfusion with $OH^{\cdot}$ generating system for subsequent estimation of MDA and the lipid metabolites.

Spectroscopic assay for MDA as TBA-reactive materials. TBA-reactive materials were estimated by the well-established technique [11]. In short, 1 ml of the perfusate was mixed with 0.2 ml of 15% trichloroacetic acid, 1 ml of 0.75% TBA in 0.5% sodium acetate was then added and mixed, and finally the mixture was boiled for 15 min [11]. The red color of the TBA–MDA complex was read with a spectrophotometer using 535 nm wavelength.

Derivatization and extraction of lipid metabolites. The lipid metabolites extracted with the perfusate were derivatized using DNPH. For derivatization purpose, 310 mg of DNPH was dissolved in 100 ml of 2 *M* HCl, and 0.1 ml of this DNPH reagent (1.56 μmol) was added to 1.5 ml of the perfusate in a 20-ml screw-capped PTFE lined test tube. An aliquot of 0.5 ml of water was added to the tube, the contents were mixed for 15 min by vortexing, and then 10 ml of pentane was added to the mixture. The tubes were intermittently shaken for 30 min, and reactions were allowed to occur at room temperature. The organic phase was removed, and the aqueous phase was extracted with an additional 20 ml of pentane. The pentane extracts were combined, evaporated under a stream of nitrogen at 30°C and reconstituted in 200 μl of acetonitrile. This acetonitrile extract was directly injected onto the HPLC column. Generation of authentic standards, calibration curves, *r*-values, and response factors were calculated as described previously [9].

HPLC procedure. A 25-μl volume of the filtered (0.2-μm Nylon-66 membrane filters in Microfilterfuge tubes from Rainin Instrument) sample was injected onto a Beckman Ultrasphere ODS C_{18} (3-μm particle size, 7.5 cm × 4.6 mm I.D.) column (Rainin Instrument) in a Waters chromatograph (Milford, MA, USA) equipped with a Model 820 full control Maxima computer system, satellite Wisp Model 700 injector, Model 490 programmable multi-wavelength UV detector (4 channels), two Model 510 pumps, and a Bondapak C_{18} Guard-Pak precolumn. The DNPH derivatives were detected at 307, 325, and 356 nm simultaneously with 3 channels of the M-490 detector at a flow-rate of 1 ml/min with an isocratic gradient of acetonitrile–water–acetic acid (40:60:0.1, v/v/v) for a total run time of 24 min. The column was washed with acetonitrile–acetic acid (100:0.1, v/v) before each day's work to remove any bound reagent.

GC–MS. In order to confirm the identity of lipid metabolities in the effluents from the heart, GC–MS analyses were performed. The GC–MS system consisted of a Hewlett-Packard Model 5890 gas chromatograph (Fullerton, CA, USA) with a 15 m × 0.32 mm I.D. capillary column 0.25 μm film thickness (Supelco SPB-5, Bellefonte, PA, USA), which was connected directly to the mass spectrometer via a heated transfer line. The transfer line temperature was maintained at 250°C. The carrier gas was helium at an average linear velocity of 65.8 cm/s, and the injection temperature was 230°C. The injector was operated in the splitless mode. A temperature program was used which consisted of a starting temperature of 75°C which was increased to 175°C at increments of 25°C/min. Between 175 and 200°C, the temperature was increased at a rate of 5°C/min and finally to 300°C at increments of 25°C/min. The mass spectrometer was a Finnigan-MAT Model 50B quadrupole instrument (Palo Alto, CA, USA) in combination with an INCOS data system. The instrument was set on electron ionization mode. The ion source temperature was 180°C, and the ionization energy was 70 ev. The system was coupled to a Data General Model DG 10 computer (Southboro, MA, USA) and a Printronix Model MVP printer (Irvine, CA, USA). For GC–MS analysis, the hydrazine derivatives of MDA, FDA, ADA, acetone and PDA were dissolved in chloroform (50 ng/μl). Similarly, hydrazine-de-

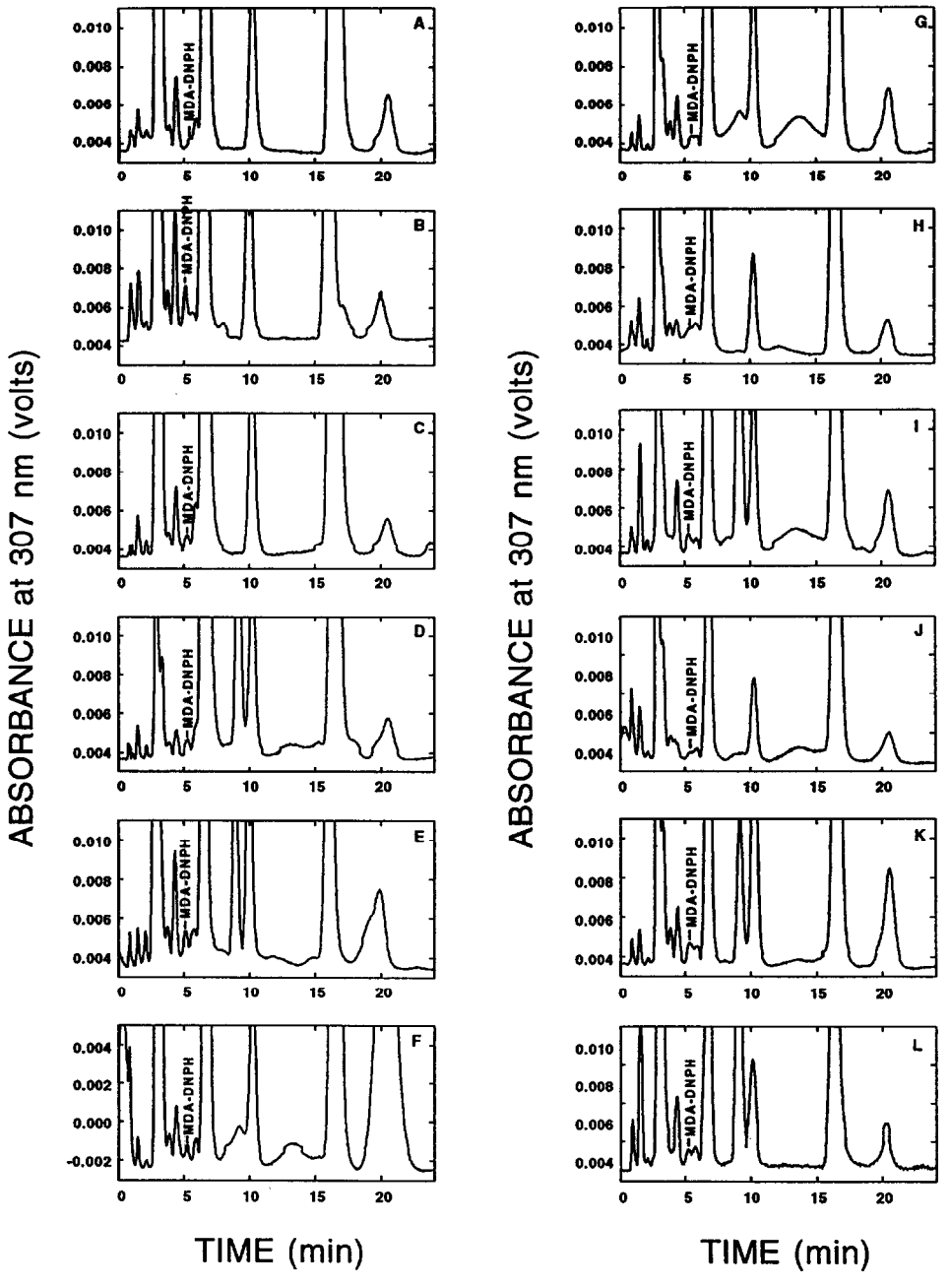

Fig. 1. Reversed-phase HPLC separation of MDA–DNPH from perfusates obtained from isolated rat hearts perfused with $OH^{\bullet}$ generating system in the presence and absence of SOD plus catalase. Perfusates were collected, derivatized and chromatographed as shown in Experimental. Absorbance was measured at 307 nm. (A) Baseline; (B) after 1 min perfusion with $OH^{\bullet}$; (C) after 10 min perfusion with $OH^{\bullet}$; (D) after 20 min perfusion with $OH^{\bullet}$; (E) after 30 min perfusion with $OH^{\bullet}$; (F) after 45 min perfusion with $OH^{\bullet}$; (G) baseline; (H) after 1 min perfusion with $OH^{\bullet}$ plus SOD and catalase; (I) after 10 min perfusion with $OH^{\bullet}$ plus SOD and catalase; (J) after 20 min perfusion with $OH^{\bullet}$ plus SOD and catalase; (K) after 30 min perfusion with $OH^{\bullet}$ plus SOD and catalase; (L) after 45 min perfusion with $OH^{\bullet}$ plus SOD and catalase.

rivatized heart perfusate samples were reconstituted in chloroform. Samples (2 μl) of standard and extracts were injected onto the GC–MS system. Following the full-spectrum identification of each of the hydrazones, a selective ion monitoring (SIM) program was developed, and additional spectra were obtained in the SIM mode.

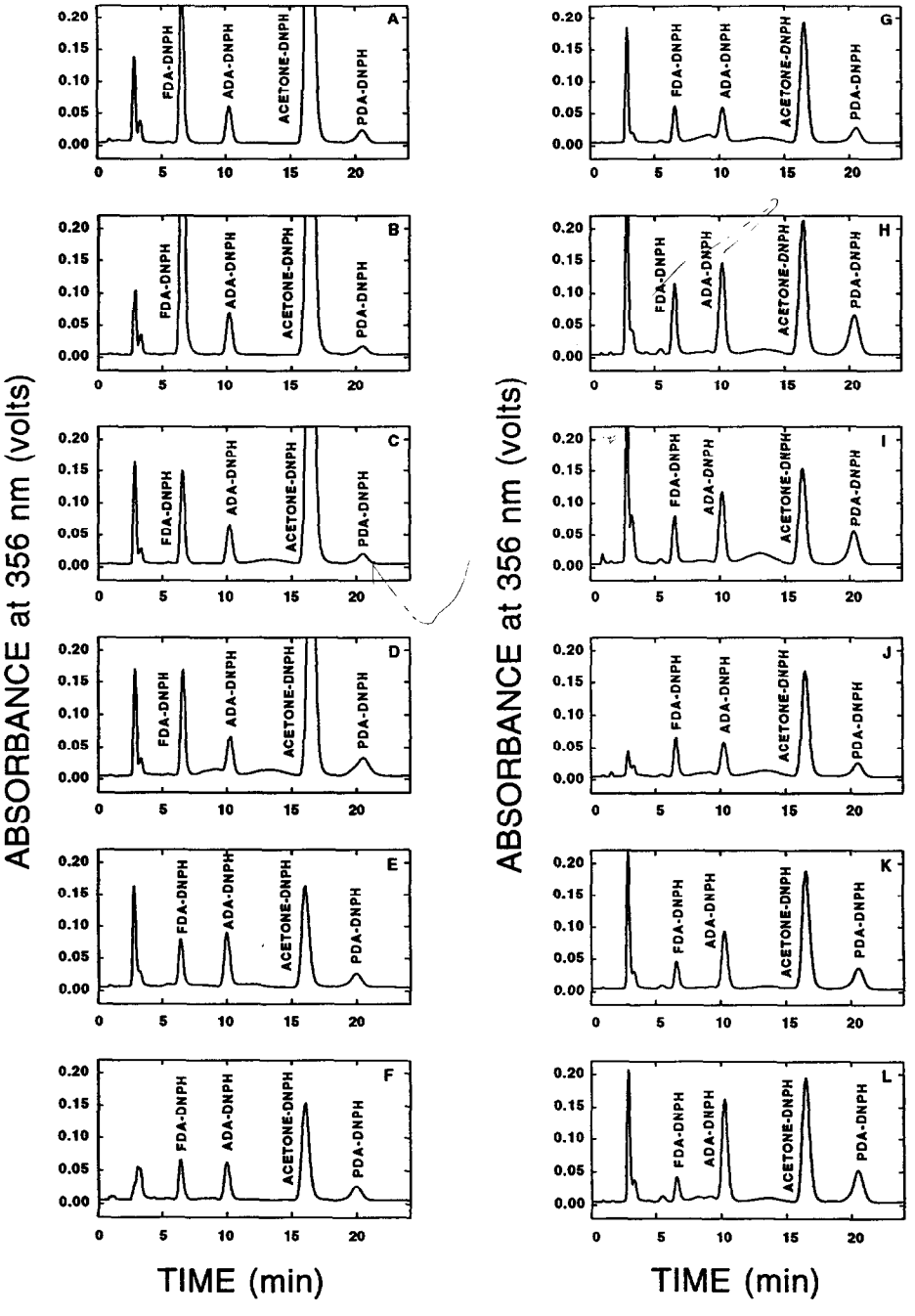

Fig. 2. Separation of FDA–DNPH, ADA–DNPH, acetone–DNPH and PDA–DNPH in rat heart perfusates under the same conditions as described in Fig. 1 except that the absorbance was measured at 356 nm (A) to (L), same as Fig. 1.

RESULTS

Separation and identification of lipid metabolites

The DNPH derivatives of authentic standards of FDA, ADA, MDA, acetone and PDA were separated using three different wavelengths: 307, 325, and 356 nm as described previously [9]. MDA gives absorption maxima at 307 nm whereas the absorption maxima for FDA, ADA, acetone and PDA is 356 nm. The retention times of MDA, FDA, ADA, acetone and PDA were 5.3, 6.6, 10.3, 16.5, and 20.5 min, respectively, making a total run time of 24 min.

Quantitative estimation of lipid metabolites in the perfusate

The perfusates obtained from the heart prior to and during the perfusion with $OH^{\cdot}$ generating system, were processed as described in Methodology, and the derivatized extracts were loaded onto the HPLC column equipped with programmable multi-wavelength UV detector. The results from the baseline (A), and after 1 min (B), after 10 min (C), after 20 min (D), after 30 min (E) and after 45 min (F) perfusion with $OH^{\cdot}$ are shown in Fig. 1 (307 nm) and Fig. 2 (356 nm). As shown in these figures, the peak for FDA, ADA, acetone and PDA are already present in the baseline samples, and they continue to be formed as the reperfusion progresses, thus causing an increase in the accumulated products. MDA peak is barely present in the baseline sample, it appears after the reperfusion, and like other lipid metabolite peaks, it continues to be formed as the duration of perfusion increases.

Each experiment was repeated by simultaneously perfusing the heart with $OH^{\cdot}$ scavengers, SOD plus catalase, in conjunction with $OH^{\cdot}$ radical generating system. As shown in Fig. 1 and Fig. 2, the peaks for the lipid metabolites either disappeared or reduced significantly after perfusing with SOD plus catalase. The exact values are shown in Table I.

In addition, spiking with the standards were also performed for each wavelength, 307 and 356 nm. Accuracy of the method was determined by standard addition technique. Addition of 50 pmol of each of the standards were accurately reflected in the peak heights. Within-run and inter-run variations were 1 and 5%, respectively.

Identification of the peaks by GC–MS

The identify of the peaks were confirmed by comparing the retention items with those of authentic standards using GC–MS. The MS data

TABLE I

PRODUCTION OF LIPID METABOLIC PRODUCTS DURING PERFUSION OF THE ISOLATED RAT HEART WITH THE $OH^{\cdot}$ GENERATING SYSTEM IN THE PRESENCE OR ABSENCE OF SOD PLUS CATALASE

Time of perfusion (min)	Perfusion agent	Amount ± S.E. ($n = 6$) (nmol/ml perfusate)				
		MDA–DNPH (307 nm)	FDA–DNPH (356 nm)	ADA–DNPH (356 nm)	Acetone–DNPH (356 nm)	PDA–DNPH (356 nm)
Baseline	$OH^{\cdot}$	0.068 ± 0.018	2.1113 ± 0.331	1.436 ± 0.177	5.015 ± 0.316	0.714 ± 0.183
	$OH^{\cdot}$ + SOD + catalase	0.053 ± 0.016	2.402 ± 0.433	1.534 ± 0.275	4.712 ± 0.431	0.684 ± 0.213
1	$OH^{\cdot}$	0.113 ± 0.030	12.684 ± 2.377	2.079 ± 0.404	8.670 ± 0.678	0.971 ± 0.258
	$OH^{\cdot}$ + SOD + catalase	0.090 ± 0.028	5.536 ± 0.809	1.753 ± 0.222	4.024 ± 0.300	0.766 ± 0.267
10	$OH^{\cdot}$	0.068 ± 0.010	6.698 ± 1.081	1.580 ± 0.216	8.651 ± 0.688	0.795 ± 0.133
	$OH^{\cdot}$ + SOD + catalase	0.053 ± 0.012	1.931 ± 0.205	1.444 ± 0.234	3.921 ± 0.213	0.593 ± 0.156
20	$OH^{\cdot}$	0.054 ± 0.005	2.189 ± 0.387	1.293 ± 0.206	5.999 ± 0.445	0.765 ± 0.136
	$OH^{\cdot}$ + SOD + catalase	0.033 ± 0.008	1.790 ± 0.221	0.756 ± 0.183	3.983 ± 0.412	0.581 ± 0.123
30	$OH^{\cdot}$	0.075 ± 0.022	2.573 ± 0.405	1.582 ± 0.345	6.973 ± 0.570	1.341 ± 0.266
	$OH^{\cdot}$ + SOD + catalase	0.065 ± 0.020	2.628 ± 0.417	1.267 ± 0.284	4.552 ± 0.131	0.826 ± 0.212
45	$OH^{\cdot}$	0.071 ± 0.023	2.217 ± 0.370	1.387 ± 0.248	6.752 ± 0.550	1.162 ± 0.078
	$OH^{\cdot}$ + SOD + catalase	0.050 ± 0.015	0.988 ± 0.156	0.856 ± 0.077	4.362 ± 0.420	0.525 ± 0.097

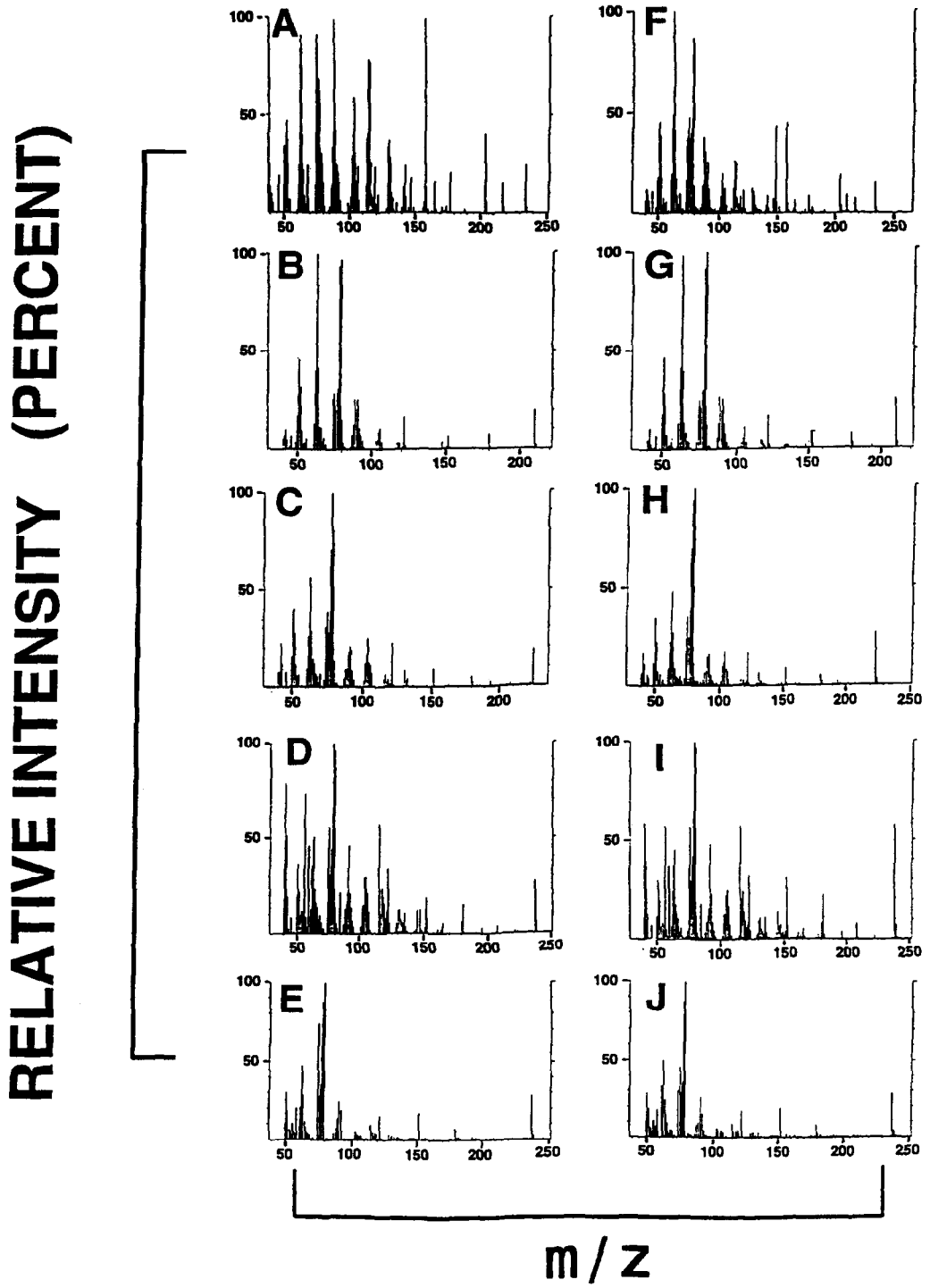

Fig. 3. Representative mass spectrum of the DNPH derivatives of the standards for the five lipid metabolites and for the five lipid metabolites in rat heart perfusates. Mass spectrum were obtained as described in Experimental. (A) MDA–DNPH standard; (B) FDA–DNPH standard; (C) ADA–DNPH standard; (D) acetone–DNPH standard; (E) PDA–DNPA standard; (F) MDA–DNPH in rat heart perfusate; (E) FDA–DNPH in perfusate; (H) ADA–DNPH in perfusate; (I) acetone–DNPH in perfusate; (J) PDA–DNPH in perfusate.

for the five lipid metabolites are presented in Fig. 3. The molecular ions (M^+) of the synthetic hydrazones of FDA, MDA, ADA, acetone, and PDA were found to be 210, 234, 224, 238, and 238, respectively (Fig. 3A–E). The same molecular ions and mass spectra were demonstrated in the extracts of heart perfusate samples following GC–MS (Fig. 3F–J).

Selective ion monitoring (SIM) was used to provide further confirmation of the identity of the five lipid metabolities in heart perfusate. Ion chromatograms for a mixture of hydrazones of the five reference standards and an extract of heart perfusate are presented in Fig. 4A–B. The ions which were selected in this display mode are 158 and 234 for MDA, 210 for FDA, 224 for ADA, 238 for acetone, and 238 for PDA. A comparison between Fig. 4A and B clearly indicates that mass ions produced by the five standards were associated with the components of heart perfusate that were separated by GC. These results provide further confirmation of the identification of the five lipid metabolites in the effluents obtained from the heart.

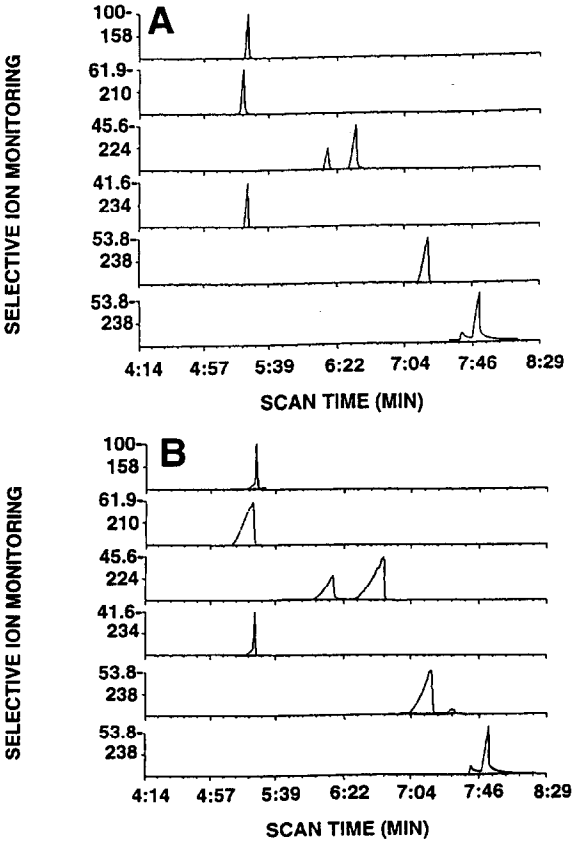

Fig. 4. Representative selective ion monitoring (SIM) for the DNPH derivatives of the standards for the five lipid metabolites and for the five lipid metabolites in rat heart perfusates. SIM obtained as shown in Experimental. (A) DNPH derivatives for standards of MDA (158, 234), FDA (210), ADA (224), acetone (238), and PDA (238). (B) DNPH derivatives of MDA (158, 234), FDA (210), ADA (224), acetone (238), and PDA (238) from rat heart perfusates.

TBA-reactive materials

The same perfusate samples were also estimated by assaying the TBA-reactive materials. The values for TBA-reactive materials are shown in Table II. As expected these values were much higher compared to those obtained for MDAs from the corresponding myocardial perfusate samples.

Correlation between the oxidative stress and the formation of lipid metabolite

To examine whether in fact these lipid metabolites truely reflect the development of oxidative stress during the perfusion with the $OH^{\bullet}$ generating system, we determined the correlation coefficient of the lipid metabolite accumulation versus the duration of $OH^{\bullet}$ perfusion. Accumulation of lipid metabolites was estimated in the perfusate obtained from heart as a function of the duration of perfusion with $OH^{\bullet}$ generating system. As shown in Fig. 5, excellent correlation was achieved for each lipid metabolite. Correlation coefficients for MDA, FDA, ADA, acetone and PDA were 0.994, 0.927, 0.997, 0.997, and 0.995, respectively, indicating the validity of monitoring these lipid metabolites for estimating oxidative stress development.

DISCUSSION

MDA and several other aldehydes and ketones, such as formaldehyde, acetaldehyde, acetone and propionaldehyde are the breakdown products of spontaneous fragmentation (β-cleavage) of peroxides derived from the free radical-polyunsaturated fatty acid (PUFA) interactions [11]. Formation of free radicals and peroxidation of PUFA are ongoing dynamic processes which

TABLE II

ESTIMATION OF MALONALDEHYDE PRODUCTION BY TBA REACTION IN HEART PERFUSATE DURING PERFUSION WITH $OH^{\cdot}$ GENERATING SYSTEM IN THE PRESENCE OR ABSENCE OF SOD PLUS CATALASE

Time of perfusion (min)	Perfusion agent	MDA–TBA ± S.E. ($n = 6$) (nmol/ml perfusate)
Baseline	$OH^{\cdot}$	0.15 ± 0.08
	$OH^{\cdot}$ + SOD + catalase	0.16 ± 0.06
1	$OH^{\cdot}$	0.16 ± 0.07
	$OH^{\cdot}$ + SOD + catalase	0.14 ± 0.07
10	$OH^{\cdot}$	0.88 ± 0.19
	$OH^{\cdot}$ + SOD + catalase	0.50 ± 0.16
20	$OH^{\cdot}$	1.45 ± 0.28
	$OH^{\cdot}$ + SOD + catalase	0.81 ± 0.19
30	$OH^{\cdot}$	2.35 ± 0.56
	$OH^{\cdot}$ + SOD + catalase	1.33 ± 0.37
45	$OH^{\cdot}$	2.67 ± 0.63
	$OH^{\cdot}$ + SOD + catalase	1.37 ± 0.24

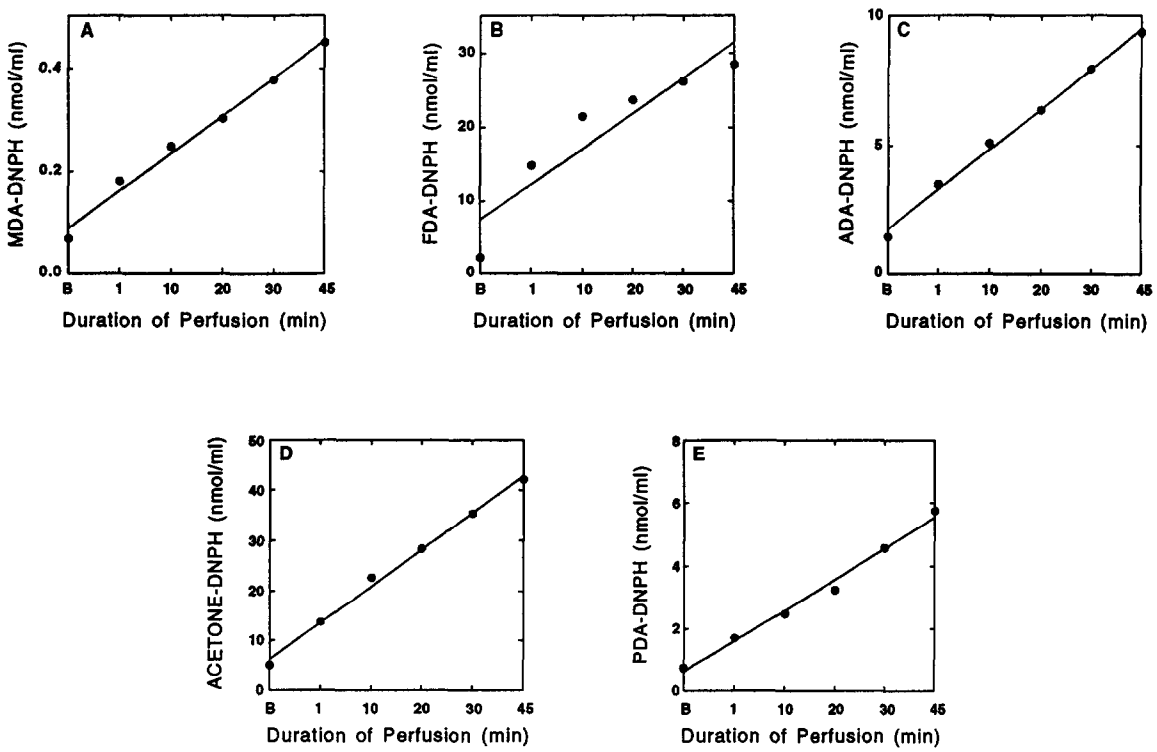

Fig. 5. Correlation of the accumulated lipid metabolites in the heart perfusates as a function of duration of perfusion with $OH^{\cdot}$ generation system. Accumulation of lipid metabolites was determined from the formation of each lipid metabolite over the total period of $OH^{\cdot}$ perfusion. (A) MDA–DNPH, correlation coefficient (r) = 0.994; (B) FDA–DNPH, $r = 0.927$; (C) ADA–DNPH, $r = 0.997$; (D) Acetone–DNPH, $r = 0.997$; (E) PDA–DNPH, $r = 0.995$.

occur in virtually all types of cells. Under normal conditions these processes are well regulated by the antioxidants and antioxidant enzymes which instantly detoxify the effect of free radicals. However, under the pathophysiological conditions as stated in the introduction, lipid peroxidation is enhanced because of the reduction in amount of tissue antioxidants in conjunction with the excessive amount of free radical production [12]. The fact that MDA represents one of the products of enzymatic PUFA oxygenation and an end product of auto-oxidative fatty peroxide decomposition and that extremely sensitive analytical methods are readily available to quantitate MDA, prompted the researchers to use MDA as presumptive marker for lipid peroxidation.

Several methods are available for the quantitation of MDA in biological tissues. These consist of direct detection or fluorometric detection of derivatized products using spectrophotometer [4,5,11] and spectrofluorometer [5,13], respectively. In addition, several methods using solvent extract followed by ion-pairing [14] or reversed-phase [15,16] HPLC and GC using nitrogen–phosphorus detection [17] or electron-capture detection [18] are also available. However, most of these methods are time-consuming and extremely sophisticated, and therefore, are not suitable for routine use in the laboratory. Among these methods, examination of TBA reactive products is the most popular and widely used to study the lipid peroxidation in biological samples. The success of the TBAR method depends on the accuracy of the determination of MDA content. However, as mentioned earlier, TBA reacts with many other compounds besides MDA, such as sugar alcohols, amino acids, glycerol and sialic acids, and as a result, it often overestimates the MDA content and yields erroneous results. This method was subsequently modified to determine MDA–TBA complex by HPLC, but the slowness as well as the complexity of the procedure could not make it easily adaptable for the routine assay of MDA.

The results of the present study demonstrate that at least five different lipid breakdown products (MDA, FDA, ADA, acetone and PDA) can be correlated with the lipid peroxidation after the development of oxidative stress in heart. In this method the carbonyls present in the myocardial tissue are converted into pyrazole and hydrazone derivatives by reacting with DNPH. This 2,4-dinitrophenyl hydrazine derivatization method in conjunction with HPLC and GC–MS was previously used to examine the lipid metabolites in the urine samples [19], and in conjunction with GC was used to examine MDA in a lipid peroxidation model system [20]. We have recently shown that this method can be used to monitor MDA formation during the reperfusion of ischemic myocardium [9]. However, we did not confirm the identity of the lipid products in this study. The present study, which is the extension of our previous observation, identified the aldehydes and ketone from the perfused myocardium by GC–MS and demonstrated an excellent correlation of the MDA values with the amount of oxidative stress, suggesting that this method is indeed suitable and highly efficient to monitor the development of oxidative stress in biological tissue such as heart.

In summary, we have shown that hydrazone derivatives of MDA and other lipid metabolites obtained from the heart subjected to oxidative stress can be assayed by HPLC as a marker for the measurement of the extent of lipid peroxidation. The simplicity, rapidity, and precision of the method should make it suitable for routine use.

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