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Original Paper

Entire Peroxidation Reaction System of Myeloperoxidase Correlates with Progressive Low-Density Lipoprotein Modifications via Reactive Aldehydes in Atherosclerotic Patients with Hypertension

Quan Liu[a] Yawen Liu[b] Jikang Shi[b] Ming Gao[a] Yunkai Liu[a,c,d] Yetong Cong[e] Yong Li[b] Yonggang Wang[a] Mingxi Yu[b] Yuping Lu[b] Dahai Wang[a] Shan Chen[a] Yang Zheng[a] Yi Cheng[a,c,d]

[a]The Cardiovascular Center, First Hospital of Jilin University, Changchun, [b]Department of Epidemiology and Biostatistics, School of Public Health of Jilin University, Changchun, [c]Key Laboratory for Cardiovascular Mechanism of Traditional Chinese Medicine, First Hospital of Jilin University, Changchun, [d]Laboratory for Cardiovascular Diseases, Institute of Translational Medicine, First Hospital of Jilin University, Changchun, [e]The General Hospital of Jilin Chemical Group Corporation, Jilin, China

Key Words
Myeloperoxidase • Reaction system • Lipoprotein • Atherosclerosis • Hypertension

Abstract
Background/Aims*:** Reactive oxygen species (ROS) contribute to the dysfunction of serum lipoproteins, which triggers lipid metabolism abnormalities in the development of atherosclerosis and hypertension. Myeloperoxidase (MPO) is involved in ROS modifications, triggering lipid peroxidation and aldehyde formation. However, the relationship between the entirety of the MPO reaction system and oxidative modification of serum lipoproteins in atherosclerotic patients with hypertension remains unclear. ***Methods: We measured MPO activity (peroxidation and chlorination), 4-hydroxynonenal-modified low-density lipoprotein (HNE-LDL), malondialdehyde-modified low-density lipoprotein (MDA-LDL), H_2O_2, reduced glutathione (GSH), and oxidized glutathione (GSSG) using a corresponding commercial kit in atherosclerotic patients with hypertension and healthy participants. We used Spearman's correlation analysis to investigate the correlation between MPO activity and the levels of these oxidative and anti-oxidative stress-related indices and performed response surface regression to investigate the relationship between the MPO reaction system and the levels of HNE-LDL, MDA-LDL, and the GSH/GSSG ratio. ***Results:*** Our results showed no association between the levels of MPO peroxidation activity, MPO chlorination activity, H_2O_2, and Cl^- and those of HNE-

Q. Liu and Y. Liu contributed equally to this work.

Yi Cheng
The Cardiovascular Center, First Hospital of Jilin University
71 Xinmin Street, Changchun, Jilin Province 130021 (China)
E-Mail chengyi@jlu.edu.cn

LDL, MDA-LDL, GSH, and GSSG, and the GSH/GSSG ratio in healthy participants. In addition, no effects of the peroxidation reaction system of MPO (PRSM) and the chlorination reaction system of MPO (CRSM) on GSH/GSSG were found in this investigation. However, we found that the PRSM rather than the CRSM correlated with progressive low-density lipoprotein (LDL) modifications by HNE-LDL and MDA-LDL in atherosclerotic patients with hypertension. ***Conclusion*:** The PRSM rather than the CRSM correlated with progressive LDL modifications via reactive aldehydes in atherosclerotic patients with hypertension. Further investigation is warranted to evaluate whether the PRSM may serve as a potential index for monitoring LDL function in atherosclerosis and hypertension.

Introduction

Cardiovascular diseases are major causes of mortality in industrialized countries, although advances have been made in the control of coronary risk factors. Abnormalities in the metabolism of lipids are involved in the development of atherosclerosis and hypertension [1]. Reactive oxygen species (ROS) contribute to the dysfunction of serum lipoproteins, thus triggering lipid metabolism abnormalities [2].

ROS generation is initiated by either nonenzymatic or enzymatic reactions. Oxidoreductases, such as myeloperoxidase (MPO) are widely expressed in neutrophils and monocytes and are a major class of enzymes that generate ROS. Neutrophils and monocytes are involved in atherogenesis and atherothrombosis because they can infiltrate the fibrous caps of ruptured plaques [3-5]. Unstable fibrous caps contain more MPO-positive cells (neutrophils and iron-containing macrophages) than stable fibrous caps. These MPO-positive cells in thrombi adjacent to disrupted plaques are also associated with occlusive thrombi [6]. MPO activates metalloproteinases, leading to the destabilization and rupture of the surfaces of atherosclerotic plaques [7, 8]. Interestingly, MPO binds to the surface of low-density lipoprotein (LDL), and causes the proatherogenic modification of LDL [9, 10]. Serum MPO levels correlate with the risk of coronary atherosclerosis [11]. Moreover, increased plasma MPO levels are observed in atherosclerotic patients [12, 13]. Plasma MPO levels are positively associated with systolic blood pressure (SBP) [14], while reductions in MPO levels correlate with the lowering of blood pressure [15]. Thus, MPO is implicated in both atherosclerosis and hypertension.

MPO catalyzes the conversion of hydrogen peroxide (H_2O_2) and chloride ion (Cl^-) to hypochlorous acid (HClO), which further generates ROS [16]. The MPO-H_2O_2-Cl^- system is involved in oxidative stress, which plays a critical role in the development of atherosclerosis and hypertension [17-20]. MPO activity is classified into peroxidation and chlorination. Interaction between MPO and H_2O_2 causes single two-electron oxidation of MPO to compound I (MPO-I), thereby activating the peroxidation activity of MPO, which oxidizes various reducible substrates. Additionally, the interaction between MPO-I and Cl^- generates HClO, which confers the chlorination activity of MPO. Importantly, H_2O_2 is essential for the peroxidation activity of MPO, H_2O_2 and Cl^- are required for the chlorination activity, and the relative levels of the reducible substrates or Cl^- determine whether MPO inclines towards peroxidation or chlorination [21]. For this reason, the MPO-H_2O_2-Cl^- system is divided into the peroxidation reaction system of MPO (PRSM) and the chlorination reaction system of MPO (CRSM). The PRSM consists of MPO peroxidation activity and H_2O_2, and the CRSM comprises MPO chlorination activity, H_2O_2, and Cl^-.

An enzyme and its substrate(s) form the reaction system of the enzyme, although enzyme activity has mostly been a target reflecting the association between the enzyme and a disease. In fact, the reaction system rather than the enzyme activity itself represents the entire function of the enzyme. However, the reaction system of an enzyme in relation to a disease, which could be a new biomarker for diagnosis, has not been thoroughly investigated. Notably, the CRSM contributes to high-density lipoprotein (HDL) oxidative damage *in vivo* [22]. The shift of the interconnection from the reaction system of paraoxonase 1 to PRSM

with HDL cholesterol (HDL-C) levels is a marker of atherosclerosis in patients with normal cholesterol levels [23]. Thus, the MPO-H_2O_2-Cl^- system could be a new comprehensive biomarker for the prediction of cardiovascular diseases.

Considering that the function of the PRSM has not been distinguished from that of the CRSM in cardiovascular diseases, and MPO is implicated in both atherosclerosis and hypertension, we investigated the association between the MPO-H_2O_2-Cl^- system (PRSM and CRSM) and the oxidative status of serum from atherosclerotic patients with hypertension in this study.

Materials and Methods

Study population

This study included 190 subjects who had no infectious diseases (93 men and 97 women) in Northeast China. Patients with stenosis were confirmed by coronary angiography. Healthy control subjects (group 1) who had no atherosclerotic coronary diseases or risk factors for atherosclerosis-related diseases were also recruited. All patients and healthy controls were selected from the First Hospital of Jilin University and the General Hospital of Jilin Chemical Group Corporation. All coronary angiograms were visually assessed by three experienced angiographers, and a consensus was reached between them. The SYNTAX score was assessed using the internet-based SYNTAX calculator (www.syntaxscore.com) [24].. The SYNTAX score is a tool for scoring the complexity of coronary artery disease, thus we selected three SYNTAX score ranges (10–22, 23–32, and ≥33), and divided the patients into groups 2, 3, or 4, respectively. Hypertension was determined by measuring blood pressure 3 times at weekly intervals (in a seated position after 5 min in a relaxed condition). Data obtained from all participants included SBP, diastolic blood pressure (DBP), Cl^-, total cholesterol (TC), HDL-C, and LDL cholesterol (LDL-C). This study was approved by the Ethics Committee of the First Hospital of Jilin University or the General Hospital of Jilin Chemical Group Corporation, and written informed consent was obtained from each participant.

Detection of laboratory parameters

Blood samples were obtained after an overnight 12-h fast. Whole blood samples were collected in Vacutainer tubes on admission. Serum was recovered after centrifugation at 4000 rpm for 5 min at 4°C. All aliquots of serum were mixed with an antioxidant solution (100 μmol/L butylated hydroxytoluene and 100 μmol/L diethylenetriaminepentaacetic acid) and stored in vacuum bags to minimize artificial oxidation at -80°C until analysis [25].

TC, HDL-C, LDL-C, and Cl^- were measured by the clinical laboratory of the First Hospital of Jilin University. Circulating 4-hydroxynonenal-modified LDL (HNE-LDL) and malondialdehyde-modified LDL (MDA-LDL) were analyzed using OxiSelect™ Human Oxidized LDL ELISA Kits (Catalog number: STA-369 and STA-389; Cell Biolabs, Inc., San Diego, CA). MPO activity was measured using Myeloperoxidase Peroxidation Assay Kit and Myeloperoxidase Chlorination Assay Kit (Item No. 700160; Item No. 10006438, Cayman Chemical Company, Ann Arbor, MI).

Total glutathione and oxidized glutathione (GSSG) were determined using the enzymatic method [26]. Reduced glutathione (GSH) is the difference between total glutathione and GSSG.

Statistical analysis

All recorded data, except SYNTAX scores, male sex, and current smoker status, were expressed as the mean ± standard deviation. The distribution of quantitative variables was tested for normality using a one-sample Kolmogorov-Smirnov test. The following quantitative variables were compared using independent-samples t tests: age, SBP, DBP, Cl^-, TC, HDL-C, LDL-C, HNE-LDL, MDA-LDL, H_2O_2, GSH, GSSG, GSH/GSSG ratio, MPO chlorination activity, and MPO peroxidation activity. Because of H_2O_2, MPO peroxidation and chlorination activities were skewed; therefore, Spearman's correlation was used to investigate any correlation between the levels of oxidative and anti-oxidative stress-related indices (HNE-LDL, MDA-LDL, GSH, GSSG, and GSH/GSSG ratio) and the levels of H_2O_2, and MPO peroxidation and chlorination activities. In addition, Pearson's correlation was used to investigate any correlation between Cl^- and the levels of the oxidative and anti-oxidative stress-related indices. Response surface regression was used to investigate the

contributions of the PRSM and CRSM to the levels of HNE-LDL, MDA-LDL, and the GSH/GSSG ratio. Two-sided *P* values < 0.05 were considered statistically significant in all analyses. Pairwise differences between the groups were assessed using a Bonferroni correction ($P < 0.016$). Analyses were performed using SPSS version 13 (SSPS Inc., Chicago, IL), Statistica version 10 (TIBCO Software, Inc., Palo Alto, CA), and Minitab version 16 (Minitab, Inc., Sydney, Australia).

Results

We investigated 45 control subjects (group 1) and 145 atherosclerotic patients with hypertension, who were divided into groups 2, 3, and 4 according to their SYNTAX scores (as described in the Methods). Smokers were present in each group, and there were more in groups 2, 3, and 4 than in group 1. However, no significant differences between group 1 and groups 2, 3, or 4 were observed ($P = 0.16$, 0.07, and 0.07, respectively). We found that TC levels in groups 2 and 4, as well as LDL-C levels in groups 2, 3, and 4, were significantly higher than in group 1 (all $P < 0.016$); TC levels in group 3 were no different from those in group 1 ($P = 0.025$); HDL-C levels were significantly lower in groups 3 and 4 than in group 1 ($P < 0.016$), but those in group 2 were no different from those in group 1 ($P = 0.023$) (Table 1).

Oxidative stress affects the pathogenesis of atherosclerosis and hypertension, thereby providing oxidative and anti-oxidative stress-related indices, including HNE-LDL, MDA-LDL, H_2O_2, GSH, GSSG, and the GSH/GSSG ratio for investigating cardiovascular diseases, [27-32]. We observed that the levels of GSH and GSSG in groups 2, 3, and 4 were no different to those in group 1. However, the levels of MDA-LDL, HNE-LDL, H_2O_2, and the GSH/GSSG ratio in groups 2, 3, and 4 were significantly different from those in group 1 (all $P < 0.016$). In agreement with the known lack of association between plasma MPO levels and the angiographic severity of coronary artery disease in patients with acute coronary syndrome [33], our results also showed that there were no significant differences in the MPO peroxidation and chlorination activities among the groups (Table 1). Moreover, the levels of the MPO peroxidation and chlorination activities, H_2O_2, and Cl^- were not associated with the levels of HNE-LDL, MDA-LDL, GSH, GSSG, or the GSH/GSSG ratio (all $P > 0.05$) (Table 2). Thus the individual components in the MPO-H_2O_2-Cl^- system did not affect the levels of the oxidative and anti-oxidative stress-related indices.

Table 1. Clinical and laboratory parameters in participants. Group 1: the healthy control participants; Group 2-4: the patients with SYNTAX score (1-22; 23-32; ≥33). SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HNE-LDL, 4-hydroxynonenal-modified LDL; MDA-LDL, malondialdehyde-modified LDL; GSH, reduced glutathione; GSSG, oxidized glutathione; MPO, myeloperoxidase. Data, except male gender, current smoker and current alcohol drinker, are expressed as mean ± standard deviation

Parameter	Group 1 (n=45)	Group 2 (n=57)	Group 3 (n=42)	Group 4 (n=46)	P Group 2 vs Group 1	P Group 3 vs Group 1	P Group 4 vs Group 1
SYNTAX score	-	10-22	23-32	≥33			
Age, years	54.3±9.1	56.6±8.5	57.3±7.9	56.5±9.3	0.191	0.105	0.257
Male gender (%)	24 (53.3)	31 (54.4)	22 (52.4)	26 (56.5)	0.925	0. 899	0.924
Current smoker (%)	15 (33.3)	28 (49.1)	25 (59.5)	25 (54.3)	0.161	0.70	0.070
SBP (mm Hg)	115.5±8.7	164.6±13.1	166.4±15.9	165.9±16.1	<0.001	<0.001	<0.001
DBP (mm Hg)	77.5±9.2	101.4±7.2	105.8±9.6	107.9±6.3	<0.001	<0001	<0.001
Cl^- (mmol/L)	102.05±2.47	102.32±2.48	102.4±3	102.45±2.53	0.585	0.552	0.447
TC (mmol/L)	4.35±0.46	5.11±1.66	5.1±1.55	5.21±1.36	0.003	0.025	0.0001
HDL-C (mmol/L)	1.23±0.31	1.09±0.3	1.07±0.13	1.02±0.43	0.023	0.002	0.009
LDL-C (mmol/L)	2.68±0.32	3.49±0.52	3.68±0.75	3.75±0.62	<0.001	<0.001	<0.001
HNE-LDL (ng/mL)	2.77±0.56	3.48±0.98	4.33±1.45	5.75±1.98	<0.001	<0.001	<0.001
MDA-LDL (ng/mL)	2.81±0.43	3.26±0.84	3.29±0.91	3.54±1	0.001	0.002	<0.001
GSH (μmol/L)	451.9±107.17	429.9±89.25	453.93±157.21	432.01±93.1	0.261	0.943	0.346
GSSG (μmol/L)	45.79±10.69	44.32±9.17	47.52±16.83	45.36±9.69	0.456	0.565	0.841
GSH/GSSG ratio	9.87±0.24	9.7±0.19	9.57±0.34	9.53±0.24	0.001	<0.001	<0.001
H_2O_2 (μmol/L)	3.38±0.7	4.09±0.96	4.37±1.07	4.19±0.95	<0.001	<0.001	<0.001
MPO peroxidation activity (ng/mL)	29.4±6.18	32.28±9.73	28.4±11.35	29.65±9.21	0.087	0.607	0.879
MPO chlorination activity (ng/mL)	23.18±1.72	23.23±1.91	23.3±1.54	23.06±1.87	0.891	0.733	0.751

Table 2. Correlation between the individual factor in MPO reaction system and the levels of oxidative and anti-oxidative stress-related indices. HNE-LDL, 4-hydroxynonenal-modified LDL; MDA-LDL, malondialdehyde-modified LDL; GSH, reduced glutathione; GSSG, oxidized glutathione; MPO, myeloperoxidase

Parameter	MPO peroxidation activity (ng/mL) r (P-value)		MPO chlorination activity (ng/mL) r (P-value)		H_2O_2 (μmol/L) r (P-value)		Cl^- (mmol/L) r (P-value)	
HNE-LDL (ng/mL)	-0.008	(0.911)	--0.083	(0.256)	0.019	(0.797)	0.02	(0.788)
MDA-LDL (ng/mL)	0.008	(0.912)	0.037	(0.613)	0.072	(0.322)	0.011	(0.882)
GSH (μmol/L)	-0.021	(0.769)	-0.087	(0.232)	-0.023	(0.753)	0.021	(0.772)
GSSG (μmol/L)	-0.023	(0.751)	-0.073	(0.316)	-0.014	(0.853)	0.015	(0.837)
GSH/GSSG ratio	-0.06	(0.412)	-0.06	(0.412)	-0.052	(0.477)	-0.006	(0.934)

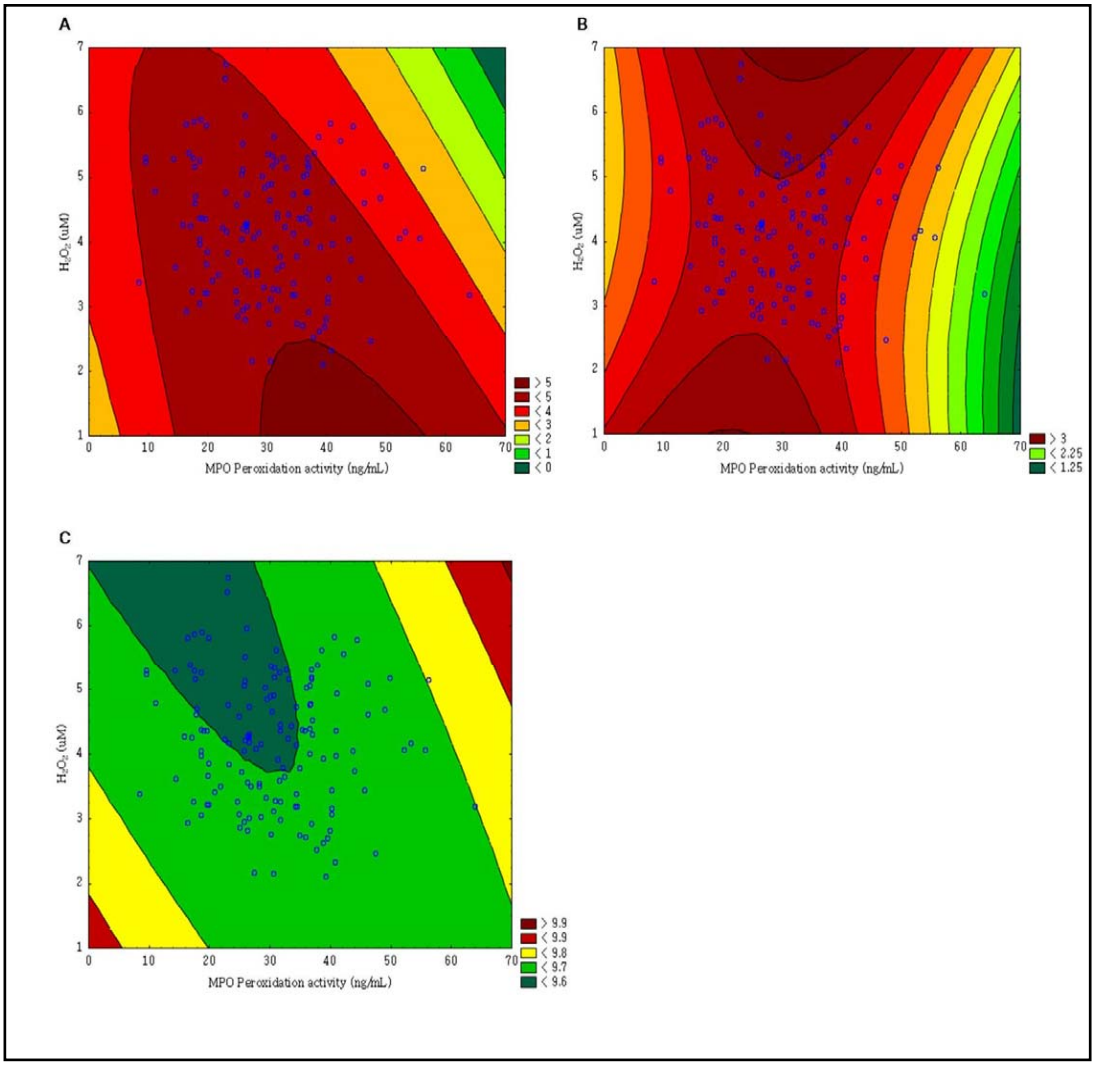

Fig. 1. Contributions of PRSM to the trend surface of HNE-LDL (A), MDA-LDL (B), and GSH/GSSG (C) in all patients.

We next focused on whether the MPO-H_2O_2-Cl^- system (PRSM and/or CRSM) is associated with the levels of HNE-LDL, MDA-LDL, or the GSH/GSSG ratio. We found that the PRSM was associated with HNE-LDL and MDA-LDL values rather than the GSH/GSSG ratio in all patients (Fig. 1). We further used response surface regression to investigate the relationships between the MPO-H_2O_2-Cl^- system and the levels of HNE-LDL, MDA-LDL, and the GSH/GSSG ratio. Our results showed no correlation between the PRSM and the levels of HNE-LDL, MDA-LDL, and the GSH/GSSG ratio in group 1 (all $P > 0.05$) (Table 3). The interaction between H_2O_2 levels

Table 3. Association between MPO reaction system and the level of HNE-LDL, MDA-LDL and GSH/GSSG ratio. Group 1: the subjects with SYNTAX score (0-5); Group 2-4: the patients with SYNTAX score (6-22; 23-32; ≥33). Coef, coefficient; SE, standard error of coefficient; MPO, myeloperoxidase; CRSM, the chlorination reaction system of MPO; PRSM, the peroxidation reaction system of MPO; MPO, myeloperoxidase; GSH, reduced glutathione; GSSG, oxidized glutathione ; HNE-LDL, 4-hydroxynonenal-modified LDL; MDA-LDL, malondialdehyde-modified LDL. * represented interaction

Group	Reaction system	HNE-LDL			MDA-LDL			GSH/GSSG ratio		
		Coef±	SE	P	Coef±	SE	P	Coef±	SE	P
1	CRSM									
	MPO Chlorination activity (ng/mL)	-0.15±	0.22	0.489	-0.1±	0.16	0.541	-0.03±	0.04	0.76
	H_2O_2 (μmol/L)	0.26±	0.3	0.404	0.04±	0.23	0.871	-0.02±	0.13	0.861
	Cl^- (mmol/L)	-0.14±	0.26	0.585	-0.15±	0.19	0.436	0. 12±	0.11	0.257
	MPO Chlorination activity * H_2O_2	-0.04±	0.41	0.916	-0.18±	0.31	0.566	-0.19±	0.17	0.273
	MPO Chlorination activity* Cl^-	-0.21±	0.3	0.497	-0.32±	0.23	0.168	-0.16±	0.13	0.217
	H_2O_2 * Cl^-	-0.18±	0.48	0.709	-0.7±	0.36	0.056	-0.03±	0.2	0.876
	PRSM									
	MPO Peroxidation activity (ng/mL)	0.5±	0.29	0.095	0.2±	0.23	0.387	-0.11±	0.13	0.381
	H_2O_2 (μmol/L)	0.14±	0.29	0.618	-0.13±	0.22	0.556	0.1±	0.12	0.419
	MPO Peroxidation activity * H_2O_2	0.75±	0.47	0.12	-0.09±	0.37	0.813	0.25±	0.2	0.223
2	CRSM									
	MPO Chlorination activity (ng/mL)	-0.15±	0.32	0.641	0.2±	0.29	0.49	-0.02±	0.07	0.721
	H_2O_2 (μmol/L)	-0.17±	0.28	0.536	0.43±	0.25	0.095	0.03±	0.06	0.776
	Cl^- (mmol/L)	-0.24±	0.3	0.427	-0.22±	0.27	0.416	0.12±	0.11	0.26
	MPO Chlorination activity * H_2O_2	-0.33±	0.51	0.522	0.12±	0.47	0.792	0.08±	0.12	0.472
	MPO Chlorination activity* Cl^-	-0.39±	0.55	0.485	-0.07±	0.5	0.889	0.05±	0.08	0.568
	H_2O_2 * Cl^-	0.16±	0.48	0.738	0.46±	0.44	0.299	0.03±	0.1	0.773
	PRSM									
	MPO Peroxidation activity (ng/mL)	0.28±	0.36	0.439	-0.32±	0.13	0.34	0.04±	0.08	0.63
	H_2O_2 (μmol/L)	0.27±	0.27	0.328	0.46±	0.25	0.067	-0.02±	0.06	0.731
	MPO Peroxidation activity * H_2O_2	1.94±	0.77	0.016	0.3±	0.7	0.673	-0.16±	0.17	0.338
3	CRSM									
	MPO Chlorination activity (ng/mL)	-0.42±	0.99	0.669	0.9±	0.58	0.128	-0.11±	0.22	0.623
	H_2O_2 (μmol/L)	-0.08±	0.654	0.901	0.61±	0.38	0.118	-0.1±	0.15	0.507
	Cl^- (mmol/L)	-0.43±	0.66	0.52	0.42±	0.39	0.283	0.02±	0.15	0.865
	MPO Chlorination activity * H_2O_2	0.57±	1.5	0.705	0.57±	0.88	0.522	0.19±	0.34	0.572
	MPO Chlorination activity* Cl^-	0.2±	1.46	0.894	0.14±	0.86	0.873	0.07±	0.33	0. 821
	H_2O_2 * Cl^-	0.06±	0.87	0.942	0.01±	0.51	0.979	-0.35±	0.2	0.087
	PRSM									
	MPO Peroxidation activity (ng/mL)	-0.52±	0.48	0.281	-0.18±	0.3	0.548	-0.04±	0.12	0. 754
	H_2O_2 (μmol/L)	-0.61±	0.52	0.254	0.61±	0.33	0.073	0.01±	0.13	0.946
	MPO Peroxidation activity * H_2O_2	-2.94±	1.28	0.027	1.7±	0.81	0.042	0.48±	0.32	0.166
4	CRSM									
	MPO Chlorination activity (ng/mL)	-0.1±	1.6	0.95	0.46±	0.89	0.608	0.21±	0.2	0.322
	H_2O_2 (μmol/L)	-0.42±	1.29	0.745	0.16±	0.71	0.825	0.1±	0.16	0.531
	Cl^- (mmol/L)	-1.9±	1.52	0.220	-1.36±	0.83	0.111	-0.11±	0.19	0.591
	MPO Chlorination activity * H_2O_2	-0.3±	1.5	0.842	-0.33±	0.82	0.695	0.26±	0.19	0.178
	MPO Chlorination activity* Cl^-	-0.09±	2.82	0.975	0.77±	1.55	0.624	0.5±	0.36	0.168
	H_2O_2 * Cl^-	2.7±	2.4	0.266	1.66±	1.31	0.214	-0.07±	0.3	0.813
	PRSM									
	MPO Peroxidation activity (ng/mL)	-0.67±	0.74	0.369	-0.49±	0.4	0.23	0.06±	0.1	0.519
	H_2O_2 (μmol/L)	-1.83±	0.62	0.005	-0.71±	0.33	0.038	0.08±	0.08	0.335
	MPO Peroxidation activity * H_2O_2	-1.5±	1.3	0.256	-1.17±	0.7	0.103	-0.04±	0.17	0.821

and MPO peroxidation activity based on the PRSM was significantly associated with HNE-LDL levels in group 2 ($P = 0.016$). Moreover, the interaction between H_2O_2 levels and the MPO peroxidation activity in the PRSM was significantly associated with the levels of HNE-LDL and MDA-LDL in group 3 ($P = 0.027$ and 0.042, respectively). Intriguingly, H_2O_2 levels, rather than the interaction between H_2O_2 levels and MPO peroxidation activity, were significantly associated with the levels of HNE-LDL and MDA-LDL in group 4 ($P = 0.005$ and 0.038, respectively) (Table 3). However, no association between the PRSM and GSH/GSSG ratio was observed in any group. The CRSM was not associated with the levels of HNE-LDL, MDA-LDL, or the GSH/GSSG ratio in any group (Table 3). As expected, the adjusted coefficients of determination (r^2-adj) of the CRSM remained zero in each group. Neither the PRSM nor the CRSM were related to the GSH/GSSG ratio (r^2-adj = 0) (Table 4). Strikingly, the PRSM contributed to

the increasing trend of the adjusted coefficients of determination (r^2-adj) to the levels of HNE-LDL and MDA-LDL from group 2 to group 4, indicating a correlating factor between PRSM and progressive LDL modifications through reactive aldehydes in atherosclerotic patients with hypertension.

Table 4. Contribution of MPO reaction system to the level of HNE-LDL, MDA-LDL and GSH/GSSG ratio in each group. Group 1: the subjects with SYNTAX score (0-5); Group 2-4: the patients with SYNTAX score (6-22; 23-32; ≥33). PRSM, the peroxidation reaction system of MPO; CRSM, the chlorination reaction system of MPO. Data were the adjusted coefficients of determination (r^2-adj) that were calculated using response surface regression

Parameter	Group 1		Group 2		Group 3		Group 4	
HNE-LDL								
PRSM	0	.019	0	.07	0	.083	0	.155
CRSM			0		0		0	.05
MDA-LDL								
PRSM	0	.013	0	.041	0	.066	0	.116
CRSM			0		0		0	
GSH/GSSG ratio								
PRSM			0		0		0	
CRSM			0		0		0	

Discussion

Our results established that the PRSM rather than the CRSM is associated with the levels of aldehyde-modified LDL in atherosclerotic patients with hypertension. Thus, the CRSM might not contribute to the levels of aldehyde-modified LDLs. The present study, therefore, identified differences in the effects of the PRSM and CRSM in the comorbidity of atherosclerosis and hypertension.

Previous studies have focused on either MPO protein levels or MPO activity in cardiovascular diseases. MPO protein levels predict risk in patients with acute coronary syndromes [34, 35]. However, large differences in the median concentrations of MPO create difficulties in ascertaining the normal MPO values of healthy individuals [36]. Increased MPO activity is associated with an oxidant-antioxidant imbalance that may contribute to atherosclerosis [37]. MPO, which binds to the surfaces of LDLs [9], is involved in lipid peroxidation, oxidative modification of LDL, and the formation of aldehydes *in vivo* [38-40]. Our results suggested that the function of the PRSM correlates with the levels of aldehyde-modified LDL in atherosclerotic patients with hypertension, although we found no association between any individual component in the MPO-H_2O_2-Cl^- system or the oxidative and anti-oxidative stress-related indices (HNE-LDL, MDA-LDL, GSH, GSSG, and the GSH/GSSG ratio).

PRSM is comprised of MPO and H_2O_2. H_2O_2 is an upstream molecule in the cascade of oxidative stress and initiates the generation of ROS. H_2O_2 levels positively correlate with the lipid peroxidation products of LDLs [41]. Lipid peroxidation caused by ROS generates MDA and HNE, which further modify LDL to form HNE-LDL and MDA-LDL. Moreover, MDA-LDL has been shown to be present in significantly higher levels in patients with acute coronary syndromes and stable coronary disease [42]. Consistent with the discovery that elevated H_2O_2 levels in blood plasma is a pathogenic factor in vascular organ damage in systemic hypertension [43], our data showed substantial differences in serum H_2O_2 levels between the control subjects and patients. Because the interaction between H_2O_2 levels and the MPO peroxidation activity in PRSM was significantly associated with the levels of HNE-LDL and MDA-LDL, the interaction may indicate that MPO enhances the use of H_2O_2 to counteract increasing H_2O_2 levels in all patients. However, in group 4, the substantial association between H_2O_2 levels and those of HNE-LDL and MDA-LDL suggested that MPO does not adequately mediate resistance to high H_2O_2 levels.

H_2O_2 is required for the conversion of GSH to GSSG, which is catalyzed by glutathione peroxidase. The GSH/GSSG ratios were substantially different between patients and control subjects, although the levels of GSH and GSSG were no different. However, the PRSM and CRSM did not contribute to the GSH/GSSG ratio in this study, suggesting that they may modify LDL, which binds to MPO.

Our study had several limitations. First, the formation of HClO from H_2O_2 and Cl^- is catalyzed by MPO, which is present only in phagocytic cells of the immune system (predominantly neutrophils). HClO is a powerful toxin that destroys bacteria within seconds

through halogenation and oxidation reactions. We excluded subjects with infectious diseases, and thereby possibly limited the CRSM function. However, we did not further measure HClO or chlorinated tyrosine levels to confirm that the function of CRSM was limited. Second, the low numbers of patients and healthy controls might have an impact on the level of statistical significance among some important variables. Third, because the degree of smoking affects the oxidative state, our study was not large enough to exclude the effect of smoking on the PRSM [44], although the patients in groups 2–4 showed no significant differences compared with the control participants in group 1. Fourth, the TC levels in group 3 showed no significantly different changes compared with those in group 1. This result was likely due in part to the SYNTAX score, which is a tool to score the complexity of coronary artery disease rather than to reflect TC levels. Fifth, medications taken by some patients might have affected the functions of the PRSM and CRSM.

Our investigation highlighted the conversion of H_2O_2 in sera. The conversion of H_2O_2 involves the participation of MPO, catalase, glutathione peroxidase, ferrous ion, and cuprous ion. Importantly, ferrous ion and cuprous ion convert H_2O_2 into a hydroxyl radical, which is the most reactive species generated among MDA and HNE, and the MPO-H_2O_2-Cl- system only contributes partially to H_2O_2 conversion. Moreover, MPO-I directly transformed to MPO, instead bypassing an interaction with Cl^-, indicating that the MPO peroxidation activity is more efficient and direct than MPO chlorination activity in the conversion of H_2O_2. Therefore, the PRSM, rather than the CRSM, correlated with progressive LDL modification via reactive aldehydes in atherosclerotic patients with hypertension. Further investigation is warranted to evaluate whether the PRSM may serve as a potential index that will enable LDL function to be monitored in atherosclerosis and hypertension.

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Disclosure Statement

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