

Probucol Reduces Myeloperoxidase Expression in Peripheral Blood Monocytes from Patients with Acute Coronary Syndrome

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Citation

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Abstract

Background Myeloperoxidase (MPO), mainly coming from peripheral blood monocytes (PBMs), which is the acute-phase reaction protein in acute coronary syndrome (ACS), plays an important role in atherosclerosis. Anti-inflammation effect may be one of benefits of probucol in ACS. However, whether probucol also affect MPO expression in PBMs in patients with ACS is unknown. **Methods** Human PBMs from ACS patients were treated with probucol. The MPO expression in PBMs was determined by Western-blot analysis. MPO levels in supernatants were measured by ELISA. Concentration of nitrites in cell supernatants was measured as an indicator of Nitric oxide (NO) production. Inducible nitric oxide synthase (iNOS) activity was evaluated by measuring the rate of conversion of L-U-¹⁴C arginine to citrulline. **Results** Compared with stable coronary heart disease (SCHD) group, MPO expression in PBMs with ACS were markedly increased ; probucol significantly reduced MPO expression and secretion of PBMs in patients with ACS. In the meanwhile, probucol increased NO levels in supernatants from patients with ACS without affecting iNOS activity. MPO expression in PBMs was negatively correlated with NO concentration in supernatants ($r=-0.548$, $p<.001$). However it was not correlated with plasma C-reactive protein (CRP) concentration ($r=0.488$, $p>0.05$). **Conclusions** Probucol reduced MPO expression in PBMs and increased NO concentration in supernatants without affecting iNOS activity These results suggested that antiatherogenic effects of probucol might, in part, due to the reduction of MPO expression.

INTRODUCTION

Increasing evidences have supported the notion that inflammation is involved in the atherogenesis and pathogenesis of acute coronary syndrome (ACS) [1]. Multiple lines of evidence have suggested that myeloperoxidase (MPO) might play an important role in atherosclerosis [1]. Higher plasma levels of MPO were correlated with increased incidence of coronary artery disease (CAD) [2] and cardiovascular events[3]. Patients who were deficient in MPO were less susceptible to cardiovascular diseases[4]. A functional MPO promoter polymorphism -463G/A, which altered MPO expression levels [5-6], was associated with increased incidence of CAD and severity of atherosclerosis [7-9]. Treatment with antioxidants may affect the expression of MPO in peripheral blood monocytes (PBMs) [10].

Nitric oxide (NO) plays essential bioregulatory roles in a wide range of processes [11]. Under pathological conditions, such as in inflammation and vascular diseases, rates of NO

consumption became excessive and the response to endothelium-derived relaxing factor was impaired [12-13]. MPO is a major scavenger and a marker of oxidative stress[14]. Superoxide, NO and MPO are seemed to be coupled through complex and inter-dependent pathway[15]. The biological consequence of NO-peroxidase interactions may have broad implication for regulation of inflammatory and cardiovascular events in vivo[16].

Probucol is a known lipophilic antioxidant with modest lipid-lowering properties. Among the antioxidants relevant to cardiovascular disease, probucol is of particular interest , as it inhibits atherosclerosis in hypercholesterolemic rabbits[17,18], non-human primates[19], and humans[20], probucol also reverses established plaques in rabbits[21] and xanthomas[22] in humans and it effectively inhibits intimal thickening and restenosis [23,24], preserves endothelial function[25]it inhibits atherosclerosis in some animals and can decrease the complications of cardiovascular disease in patients with

hypercholesterolemia[26]. In the Fukuoka Atherosclerosis Trial (FAST), probucol lowered cholesterol and stopped progression of atherosclerotic plaques in carotid arteries [27]. Our previous study had shown that probucol up-regulated paraoxonase 1 expression in hypercholesterolemic rabbits, which might be contributed to its antiatherogenic effect [28]. Paul et al [29] showed that probucol protected against HOCL-induced endothelial dysfunction. However, the exact mechanisms underlying its vascular protective activity were remained unclear. Therefore we decided to test the effect of probucol on the expression of MPO in PBMs from patients with ACS and SCHD, and to examine whether the benefit activity of probucol was related to another inflammatory factor-MPO.

MATERIALS AND METHODS

PATIENTS

We recruited 35 patients with acute coronary syndrome (ACS group) including acute myocardial infarction and unstable angina. 20 patients with stable coronary heart disease (SCHD group) of matched age were treated as control. Those patients with unstable angina had experienced ischemic chest pain at rest accompanied with transient ST-T segment depression and/or T-wave inversion within the proceeding 24 h. The diagnosis of acute myocardial infarction was based on a history of ischemic chest pain > 30 min, characteristic ECG changes, and increasing of creatine kinase activities at least twice the upper normal level within 24 h after the onset of pain. Stable CHD was defined as clearly established CHD with no history of recent hospital admission (within the previous 3 months) for treatment of a coronary condition. All patients underwent coronary angiography, and Gensini scores were used to evaluate coronary severity. Exclusion criteria included body temperature > 38°C, inflammatory diseases (e.g., infections, malignancies, autoimmune diseases), impaired liver function, renal failure, serum total cholesterol concentration > 7.0 mmol/L, and recent major surgery. PBMs were collected from all participants, and the monocytes from patients with ACS were collected within 12 h after the onset of pain. Serum levels CRP as well as lipid profiles, liver and kidney function, cardiac enzyme (CK and CK-MB) were obtained in the fasting state the first day. All the venous blood samples were drawn into pyrogen-free blood collection tubes without additive. The serum was collected after centrifugation at 3500 rpm for 3 min and then stored at -70°C until analyzed. Serum CRP levels were determined by turbidimetric immunoassay (CRP kit, Beckman Coulter),

and the examinations were done in Array 360 Analyzer (Beckman Coulter). Total cholesterol (TC) and triglyceride (TG) concentrations were analyzed using a Hitachi 747 autoanalyzer (Boehringer Mannheim, Naka, Japan). Serum glucose was measured using the glucose oxidase method. MPO levels in plasma were measured by an ELISA test kit (Corgenix, Inc, USA). The study was approved by the local Institutional Review Board.

PBMS ISOLATED AND CULTURE

Peripheral venous blood from the patients with ACS (15 mL) or with stable CHD (5 mL) was layered on an equal volume of Ficoll Hypaque (Shanghai Biotech) and was centrifuged at 1500g for 20 min at 25 °C. PBMs were harvested from the Ficoll-plasma interface and were washed twice with cold Hanks' balanced salt solution (Sigma) and then once with serum-free RPMI 1640 (Gibco Life Technologies). The final pellet was suspended in serum-free RPMI 1640, and the cells were plated in 75-cm² flasks at a density 2 x 10⁷ cells/dish. Cell viability, as assessed by trypan blue exclusion, was routinely >95%. Nonadhering cells were removed after 6 h of incubation. Adhering monocytes were cultured for 24 h with RPMI 1640 supplemented with 2mmol/L N-acetyl-L-alanyl-L-glutamine, 100 kilounits/L penicillin, 100 mg/L streptomycin, 20 g/L sodium pyruvate, 20 mmol/L HEPES (Gibco Life Technologies), and 100 mL/L heat-inactivated fetal calf serum (Gibco Life Technologies) at 37°C in 5% CO₂. Cell culture supernatants were collected and stored at -70°C for MPO assays. Adhesive monocytes were used to extract total protein for Western blot. For in vitro studies, PBMs from patients with ACS were isolated as described above. After removal of the nonadhering cells, monocytes were exposed to probucol (0.5-10 μmol/L) dissolved in dimethyl sulfoxide (Sigma) for 24 h, and cells were collected for MPO assays, and supernatants were collected for detection of MPO levels, NO concentration, as well as iNOS activity.

DETECTION OF MPO EXPRESSION IN CULTURED PBMS BY WESTERN BLOT ANALYSIS

To investigate the expression of MPO in human PBMS, immunoblot analysis were performed. Cells (2x10⁷) were lysed with Triton lysis buffer (50 mM Tris-HCL, pH 8.0 containing 150 mM NaCL, 1% Triton X-100, 0.02% sodium azid, 10 mM EDTA, 10 μg/ml aprotinin, and 1μg/ml aminoethylbenzenesulfonyl fluoride). Protein concentrations were determined by Bradford assay. One hundred

micrograms protein from cell lysates were loaded onto a 7.5% SDS-PAGE gel. After electrophoresis, the SDS-PAGE separated proteins were transferred to a nitrocellulose membrane (Amersham Pharmacia Biotech). The membrane was blocked with 2.5% nonfat milk in PBS and incubated with rabbit antibody against human MPO(1:200 dilution) in PBS for 2 h. Then the membrane was incubated with goat anti-rabbit IgG conjugated with horseradish peroxidase (Santa Cruz, Biotechnology, CA, USA) at 1:1000 in PBS for 1h. Blots were processed using an ECL kit (Santa Cruz) and exposed to X-ray film.

BIOCHEMICAL ANALYSIS

MPO levels in supernatants were measured by an ELISA test kit (Corgenix, Inc, USA). Concentration of nitrites in cell supernatants was measured as an indicator of NO production according to the method of Gilliam et al. Briefly, the total concentration of nitrites was determined colorimetrically with the Griess reagent. The absorbance was measured at 546 nm (Spectra Rainbow, SLT, Austria). Sodium nitrite was used as a standard. NOS activity was evaluated by measuring the rate of conversion of L-[U-¹⁴C]arginine to citrulline, according to the method of Salter et al. The activity was expressed as nmol/min/ml of supernatant.

STATISTICS

Student's t test or analysis of variance (ANOVA) was used to determine statistical significance of probucol effects using SPSS 15.0 (SPSS software). All values are expressed as means±SEM. A probability less than 0.05 was considered significant. The association of measurements with other biochemical variables was assessed by the Spearman rank correlation test.

RESULTS

BASELINE CHARACTERISTICS OF PATIENTS WITH ACS AND SCHD

The baseline characteristics of the patients are summarized in Table 1. Sex distribution, age, body mass index, smoking, fasting blood glucose, creatinine, TG, TC, Gensini scores, LVEF and blood pressure were not significantly different among the groups; while white blood cells, PBMC-MPO expression, serum-MPO and CRP were significantly higher in ACS group as compared with SCHD group. There was significant negative correlation between PBMC-MPO expression and LVEF for all patients ($r = -0.702$, $P < 0.001$). However it was not correlated with plasma CRP concentration ($r = 0.488$, $p > 0.05$) or serum-MPO ($r = 0.287$, p

> 0.05), and no statistically significant correlation was found between PBMC-MPO expression and Gensini scores ($r = 0.160$, $P > 0.1$).

Figure 1

Table 1. Baseline characteristics of patients with acute coronary syndrome (ACS group) and stable coronary heart disease (SCHD group)

	ACS group (n=35)	SCHD group (n=20)
Age (year)	61.7±7.3	59±8.6
Male/female (n)	18/7	12/4
BMI(kg/m ²)	22.1±2.3	21.2±2.5
Smoking(n)	14(56%)	8(50%)
Hypertension (n)	10(40%)	5(31%)
Diabetes mellitus (n)	5(20%)	3(18%)
Fasting blood glucose (mmol/L)	5.44±0.34	5.43±0.22
WBC (× 10 ⁹ /L)	11.22±0.99*	8.15±0.72
Creatinine (μmol/L)	67.2±4.06	60.15±2.02
TG(mmol/L)	2.18±0.19	1.88±0.17
TC(mmol/L)	5.32±0.24	4.80±0.15
CRP (mmg/L)	30.86±2.98*	19.17±2.54
PBMC-MPO	1.24±0.12*	0.75±0.04
Plasma-MPO	36.64±2.53*	26.20±1.75
Gensini scores	22.80±2.12	21.32±1.86
LVEF (%)	55.12±2.60	53.50±2.1

As compared with SCHD group, * $p < 0.05$.

Values are presented as mean ± SEM. or n (%).

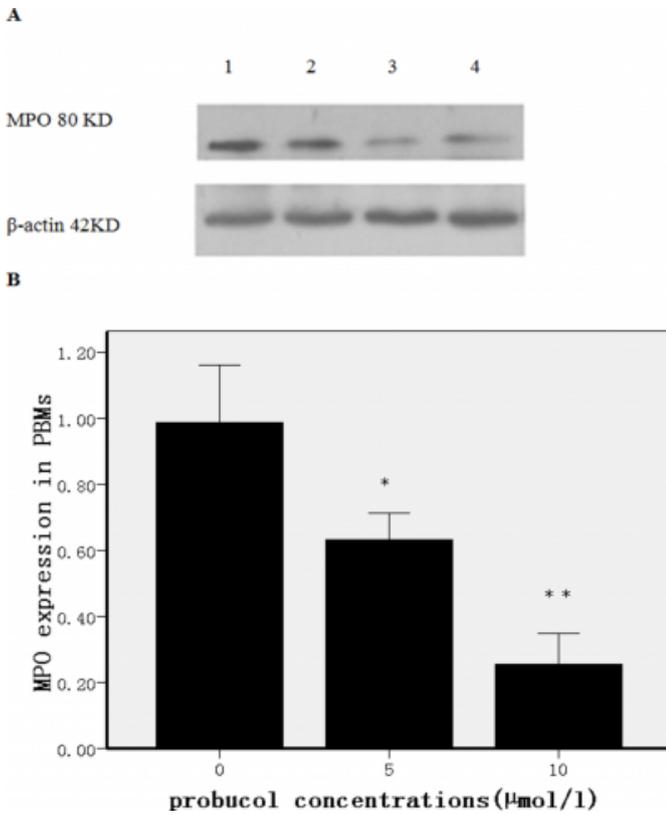
BMI: Body Mass Index; WBC: white blood cell; TG: triglyceride; TC: total cholesterol;

CRP: C Reactive Protein.

2.2 As shown in Figure 1, MPO expression in PBMs with ACS were markedly increased as compared with SCHD group (0.99 ± 0.09 vs 0.32 ± 0.04 , $p < 0.01$); probucol significantly reduced MPO expression in PBMs with ACS in a dose-dependent manner.

Figure 2

Fig. 1: Effect of probucol on MPO expression in peripheral blood monocytes from patients with ACS. Effect of probucol on MPO expression in peripheral blood monocytes from patients with ACS (n=35)



A: Detection of MPO expression in cultured PBMs from patients with ACS and SCHD by Western blot analysis. Lane 1: probucol pretreatment (0μmol/L); Lane 2: probucol 5μmol/L; Lane3: probucol (10μmol/L); Lane 4: SCHD group.

B: The effects of probucol at various concentration on MPO expression in PBM with ACS. MPO expression is expressed as absorbance ratio of MPO product to β-actin product.

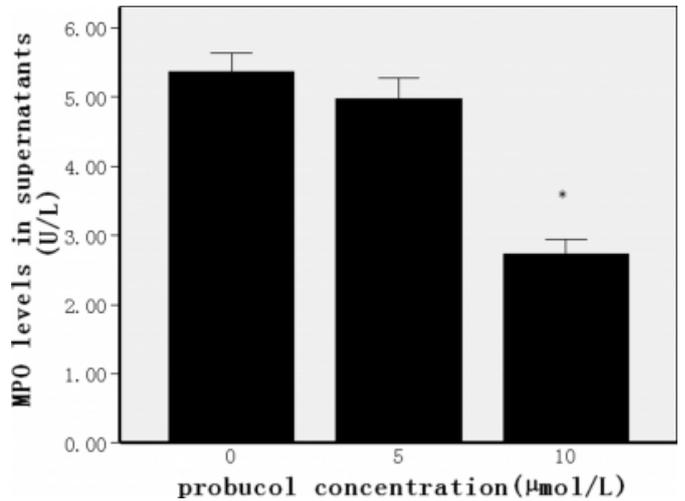
*:indicates a significant difference (p<0.01, as compared with probucol 0μmol/L group);

** indicates a significant difference (p<0.001, as compared with probucol 0μmol/L and probucol 5 μmol/l group).

2.3 MPO levels in supernatants were markedly higher in ACS group than that in SCHD group (5.65±0.18 vs 4.66±0.12 U/L, p<0.01); as shown in Figure 2, Probucol decreased MPO secretion of PBMs in ACS group in dose - dependent manner; however a significant decrease was recorded only with probucol concentration of 10 μmol/L .

Figure 3

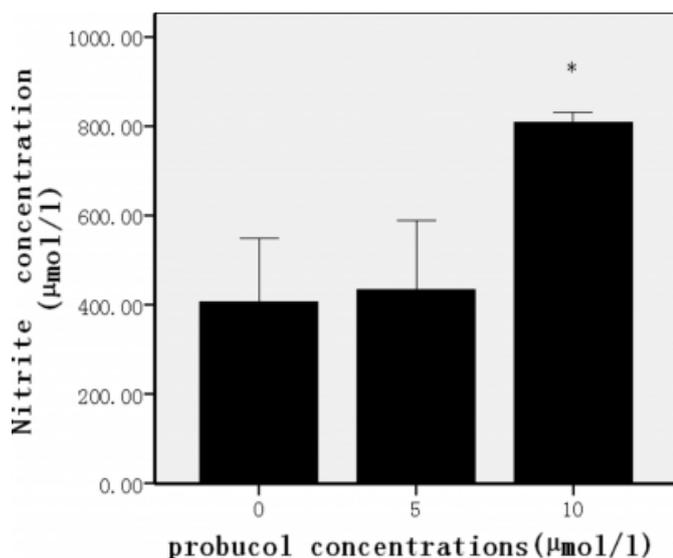
Fig 2: Effect of probucol on MPO release from patients with ACS. MPO levels in supernatants were measured by an ELISA test kit . 0: probucol concentration at 0μmol/L, 5: probucol concentration at 5μmol/L 10: probucol concentration at 10μmol/L. * indicates a significant difference (p<0.05, as compared with probucol concentration at 0μmol/L and 5μmol/L).



2.4 As shown in Fig 3, probucol dose-dependently increased NO levels in supernatants from patients with ACS without affecting iNOS activity(data not shown), however a significant increase was recorded only with probucol concentration of 10 μmol/L.

Figure 4

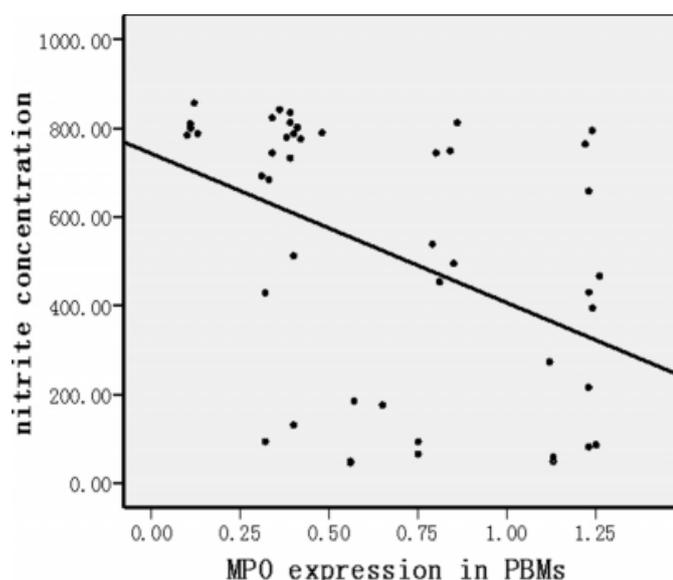
Fig 3. Effect of probucol on Nitrite concentration in supernatants from patients with ACS. 0: probucol concentration at 0 $\mu\text{mol/L}$, 5: probucol concentration at 5 $\mu\text{mol/L}$, 10: probucol concentration at 10 $\mu\text{mol/L}$. Concentration of nitrites in cell supernatants was measured as an indicator of NO production according to the method of Gilliam et al. *indicates a significant difference ($p < 0.05$, as compared with probucol concentration at 0 $\mu\text{mol/L}$ and 5 $\mu\text{mol/L}$).



2.5 As shown in Fig 4, MPO expression in PBMs from patients with ACS was negatively correlated with NO concentration in supernatants ($r = -0.548$, $p < 0.001$).

Figure 5

Fig.4 The correlation of nitrite concentration and MPO expression in PBMs.



MPO expression in PBMs is expressed as absorbance ratio

of MPO product to β -actin product; concentration of nitrites in cell supernatants was measured as an indicator of NO production according to the method of Gilliam et al. $r = -0.548$, $p < 0.001$.

DISCUSSION

Local and systemic inflammatory responses are commonly involved in the progress of ACS. PBMs are activated after ACS, and these inflammatory cells are important in blood prone to thrombosis (vulnerable blood). MPO which is largely abundant expressed in neutrophils and monocytes catalyzes the formation of many reactive oxidant species [30-31]. MPO promotes atherogenesis via a range of mechanisms. One of the major mechanisms is that MPO can utilize the nitric oxide (NO) as substrate to generate NO-derived oxidants, which leads to endothelial dysfunction, accumulation of foam cells in the arterial wall and the promotion of plaque rupture [32]. Evidence suggests that MPO-generated reactive nitrogen species can convert native low density lipoprotein (LDL) into an atherogenic form, promote the peroxidation of lipids [33]. It is also appeared that MPO can selectively modify apolipoprotein A-I, impair ABCA-1 dependent cholesterol efflux [34]. Moreover, studies suggest MPO plays a potential role in plaque destabilization and rupture due to activation of matrix metalloproteases (MMPs). In addition to, Sugiyama et al.[35] demonstrated that HOCl could augment tissue factor (TF) expression in human EC and provoke human EC death and detachment through both apoptotic and oncotic cell death pathways. Their results suggested that MPO-derived HOCl might participate in ACS by promoting superficial erosion and increasing thrombogenicity. In our study, PBMC- MPO expression and MPO levels in supernatants were significantly higher with ACS as compared those with SCHD, while Gensini scores were not significantly different among these two groups, which is consistent with the results of other authors, indicate that MPO takes part in the acute complication phases of the atherosclerotic process; and there was significant negative correlation between PBMC-MPO expression and LVEF for all patients ($r = -0.702$, $P < 0.001$), indicate that MPO may impaired heart function. As a result, MPO and its downstream inflammatory pathways may become attractive targets for both prognostication and therapeutic intervention in the atherosclerotic cardiovascular disease.

Probucol was originally used as hypolipidemic drug to prevent atherosclerosis, due to its inhibitory effect on the

oxidation of LDL. Recently, several studies suggested that probucol might also have anti-inflammatory actions to explain its anti-atherosclerotic activity by reducing vascular cell adhesion molecule-1 and monocyte chemoattractant protein-1[36], up-regulating paraoxonase-1 expression[27] and inducing heme oxygenase-1 paraoxonase-1 expression [37]. In the present study, we demonstrated, for the first time, probucol can down-regulate MPO expression in PBMs and reduced the MPO levels in supernatants with ACS. Though there were some discrepancies between the MPO expression in PBMs and the secretion into the supernatant among ACS patients, these discrepancies may be due to the concentrations of probucol, at 10 μ mol/L or higher, it inhibits both expression and secretion. Accordingly, probucol significantly increases NO levels in PBMs from patients with ACS without affecting iNOS. The MPO expression in PBMs from patients with ACS is negatively correlated with NO levels in supernatants, indicating that probucol reduces NO removal by MPO, and it can not produce more NO from monocytes (because without affecting iNOS). All of these results suggest that probucol may play anti-atherosclerosis effects by reducing MPO expression in PBMs. It is not known how probucol could modify the expression of MPO in monocytes. It has been suggested that some inflammatory genes transcription and expression are regulated through an antioxidant-sensitive mechanism in HUVEC[38]. By actively inhibiting NF- κ B mobilization, antioxidants like NAC or PDTC can reduce the expression of some inflammatory factors, such as VCAM-1, TNF- κ B, IL-1 or MPO[39,40,41], probucol, a potent antioxidant, may also suppress inflammatory factors through the same mechanism. Recent evidence indicates that agonists for the peroxisome proliferator-activated receptor (PPAR) regulate MPO gene expression via the aforementioned ALuRRE[42,43], while our laboratory's previous study reported that probucol suppressed NF- κ B-p65 expression induced by Ang II, along with increases in the expression of PPAR. Therefore, the mechanism which probucol reduced the MPO expression and secretion is probably related to NF- κ B or PPAR[44].

ACS might relate to monocytes activation and MPO increasing. In our present study, MPO expression in PBMs with ACS were markedly increased, probucol can decrease MPO expression and secretion in PBMs in patients with ACS, it may also decrease MPO levels in serum. Therefore, treatment with probucol may be a new way in ACS.

In conclusion, we have been able to show a novel target site for the protective action of probucol, in which this drug can reduce MPO expression in PBMs with ACS. Further clinical studies will be required to substantiate whether probucol may influence on plasma MPO levels with ACS patients and whether treatment with probucol can improve heart function.

References

1. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135-1143.
2. Zhang R, Brennan ML, Fu X, et al. Association between myeloperoxidase levels and risk of coronary artery disease. *JAMA* 2001, 286: 2136-2142.
3. Baldus S, Heeschen C, Meinertz T, et al. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. *Circulation* 2003, 108:1440-1445.
4. Milla C, Yang S, Cornfield DN, et al. Myeloperoxidase deficiency enhances inflammation after allogeneic marrow transplantation. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2004 Oct;287(4):L706-14.
5. Kutter D, Devaquet P, Vanderstocken G, et al. Consequences of total and subtotal myeloperoxidase deficiency: risk or benefit?, *Acta Haematol.* 2000,104 : 10-15.
6. Nikpoor B, Turecki G, Fournier C, et al. A functional myeloperoxidase polymorphic variant is associated with coronary artery disease in French-Canadians. *Am. Heart J.* 2001,142: 336-339.
7. Asselbergs FW, Reynolds WF, Cohen-Tervaert JW, et al. Myeloperoxidase polymorphism related to cardiovascular events in coronary artery disease. *Am. J. Med.* 2004,116: 429-430
8. Makela R, Karhunen PJ, Kunnas TA, et al. Myeloperoxidase gene variation as a determinant of atherosclerosis progression in the abdominal and thoracic aorta: an autopsy study. *Lab. Invest.* 2003,83:919-925.
9. Makela R, Laaksonen R, Janatuinen T, et al, Myeloperoxidase gene variation and coronary flow reserve in young healthy men. *J. Biomed. Sci.* 2004;11:59-64
10. Oram JF, Yokoyama S. Apolipoprotein-mediated removal of cellular cholesterol and phospholipids. *J Lipid Res* 1996;37:2473-2491.
11. Fleming I, Busse R. The physiology of nitric oxide: control and consequences. *Curr Med Chem-Anti-Inflammatory & Antiallergy Agents* 2004;3:89-205
12. Dessy C, Feron O. Pathophysiological roles of nitric oxide: in the heart and coronary vasculature. *Curr Med Chem-Anti-Inflammatory & Antiallergy Agents* 2004;3:207-216
13. Wu CC. Nitric oxide and inflammation. *Curr Med Chem-Anti-Inflammatory & Antiallergy Agents* 2004;3:217-222
14. Brennan ML, Hazen SL. Emerging roles of myeloperoxidase and oxidant stress markers in cardiovascular risk assessment. *Curr Opin Lipidol* 2003;4:353-359
15. Abu-Soud HM, Hazen SL. Nitric oxide modulates the catalytic activity of MPO. *J Biol Chem* 2000;275:5425-5430
16. Abu-Soud HM, Hazen SL. Nitric oxide is a physiological substrate for mammalian peroxidase. *J Biol Chem* 2000;275:37524-37532
17. Kita, T., Nagano, Y., Yokode, M., Ishii, K., Kume, N.,

- Ooshima, A., Yoshida, H., and Kawai, C. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Proc. Natl. Acad. Sci. U. S. A.* 1987; 84, 5928–5931
18. Carew TE, Schwenke DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Proc Natl Acad Sci U S A.* 1987 Nov;84(21):7725–7729.
19. Sasahara M, Raines EW, Chait A, Carew TE, Steinberg D, Wahl PW, Ross R. Inhibition of hypercholesterolemia-induced atherosclerosis in the nonhuman primate by probucol. I. Is the extent of atherosclerosis related to resistance of LDL to oxidation? *J Clin Invest.* 1994 Jul; 94(1):155–164.
20. Sawayama, Y., Shimizu, C., Maeda, N., Tatsukawa, M., Kinukawa, N., S., Kashiwagi, S., and Hayashi, Effects of probucol and pravastatin on common carotid atherosclerosis in patients with asymptomatic hypercholesterolemia. *J. Am. Coll. Cardiol.* 2002; 39:610-616
21. Nagano Y, Nakamura T, Matsuzawa Y, Cho M, Ueda Y, Kita T. Probucol and atherosclerosis in the Watanabe heritable hyperlipidemic rabbit—long-term antiatherogenic effect and effects on established plaques. *Atherosclerosis.* 1992 Feb;92(2-3):131–140
22. Kajinami K, Nishitsuji M, Takeda Y, Shimizu M, Koizumi J, Mabuchi H. Long-term probucol treatment results in regression of xanthomas, but in progression of coronary atherosclerosis in a heterozygous patient with familial hypercholesterolemia. *Atherosclerosis.* 1996 Feb;120(1-2):181–187
23. Ferns GAA, Forster L, Steward-Lee A, Konneh M, Nourooz-Zadeh J, Änggård EE. Probucol inhibits neointimal thickening and macrophage accumulation after balloon injury in the cholesterol-fed rabbit. *Proc Natl Acad Sci U S A.* 1992;89:11312–11316
24. Tardif J-C, Côté G, Lespérance J, Bourassa M, Lambert J, Doucet S, Bilodeau L, Nattel S, de Guise P. Probucol and multivitamins in the prevention of restenosis after coronary angioplasty. *N Engl J Med.* 1997;337:365–372.
25. Effect of cholesterol lowering and anti-oxidant therapy on endothelium-dependent coronary vasomotion. *N Engl J Med.* 1995;332:488–493
26. Yamamoto A, Matsuzawa Y, Yokoyama S et al. Effects of probucol on xanthomata regression in familial hypercholesterolemia. *Am J Cardiol* 1986;57 :29H–35H.
27. Tardif JC, Gregoire J, Schwartz L et al. Effects of AGI-1067 and probucol after percutaneous coronary interventions. *Circulation* 2003;107: 552–558.
28. Hong SC, Zhao SP, Wu ZH. Probucol up-regulates paraoxonase-1 expression in hepatocytes of hypercholesterolemic rabbits. *J cardiovasc pharmacol* 2006;47(1):77-81
29. Paul KW, Ben JW, Mark R et al. Probucol protects against hypochlorite-induced endothelial dysfunction. *J Bio Chem* 2005;280(16):15612-15618
30. Abu-Soud HM, Hazen SL. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *J. Biol. Chem.* 2000; 275:37524–37532.
31. Eiserich JP, Cross CE, Jones AD, et al., Formation of nitrating and chlorinating species by reaction of nitrite with hypochlorous acid. A novel mechanism for nitric oxide-mediated protein modification. *J Biol Chem* 1996;271:19199–19208
32. Hazen SL. Myeloperoxidase and plaque vulnerability. *Arterioscler Thromb Vasc Biol* 2004;24:1143–1146.
33. Nicholls SJ, Hazen SL. The role of myeloperoxidase in the pathogenesis of coronary artery disease. *Jpn J Infect Dis* 2004;57: S21–S22.
34. Zheng L, Nukuna B, Brennan ML, et al. Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J Clin Invest* 2004;114: 529–541
35. Sugiyama S, Kugiyama K, Aikawa M, et al. Hypochlorous acid, a macrophage product, induces endothelial apoptosis and tissue factor expression. Involvement of myeloperoxidase-mediated oxidant in plaque erosion and thrombogenesis. *Arterioscler. Thromb. Vasc. Biol.* 2004 Jul;24(7):1309-14.
36. Sundell CL, Somers PK, Meng CQ, et al. AGI-1067: a multifunctional phenolic antioxidant, lipid modulator, anti-inflammatory and antiatherosclerotic agent. *J pharmacol Exp Ther* 2003;305:1116-1123
37. Deng YM, Wu BJ, Witting PK, et al. Probucol protects against smooth muscle proliferation by upregulating heme oxygenase-1. *Circulation* 2004;110:185-1860
38. P. Alan. F. Kumar and R. Wanda. Statins downregulate myeloperoxidase gene expression in macrophages. *Biochem Biophys Res Commun* 2005;331:442-451
39. Tao Zhou, Shen-hua Zhou, Shu-shan Qi, et al. The effect of Atorvastatin on serum myeloperoxidase and CRP levels in patients with acute coronary syndrome. *Clin Chim Acta* 2006 Jun 368;(1-2):168-72
40. Marui N, Offermann NK, Swerlick R, et al. Vascular cells adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 1994;92:1866-74
41. Martina Z, Paul G.F, Marzia B, et al. Disruption of the aspartate to heme ester linkage in human myeloperoxidase: IMPACT ON LIGAND BINDING, REDOX CHEMISTRY, AND INTERCONVERSION OF REDOX INTERMEDIATES *J Biol Chem.* 2007;282:17041-17052
42. Alan P. Kumar, F. Javier P, et al. Peroxisome proliferators-activated receptor gamma ligands regulate myeloperoxidase expression in macrophages by an estrogen-dependent mechanism involving the -463GA promoter polymorphism *J Biol Chem.* 2004;279:8300-8315
43. Wanda FR, Alan P, Kumar F, Javier P. The human myeloperoxidase is regulated by RXR and PPAR alpha ligands *Biochem Biophys Res Commun* 2006;349:846-854
44. Zhan M, Zhou Sh-H, Liu QM, et al. The effects of Angiotensin II on Bone Morphogenetic Proteins-2, NF-κB-p65 expression in Human Umbilical Vein Cells *Chinese Journal of hypertension* 2006;14(12):1007-1010

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