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Homocysteine induces production of monocyte chemoattractant protein-1 and interleukin-8 in cultured human whole blood[1]

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KEY WORDS atherosclerosis; monocyte chemoattractant protein-1; interleukin-8; homocysteine; monocytes

ABSTRACT

AIM: To investigate whether increased plasma *L*-homocysteine (Hcy) level could promote monocyte chemoattractant protein-1 **(**MCP-1) and interleukin-8 (IL-8) in cultured whole blood. **METHODS**: Human whole blood or different type of peripheral blood cells from health volunteers were incubated with Hcy and/or the inhibitors. MCP-1 and IL-8 level were measured by ELISA assay. **RESULTS:** Hcy 10-1000 μmol/L induced production of MCP-1 and IL-8 in cultured human whole blood ($P<0.05$). The major cellular source of these chemokines comed from monocytes. Meanwhile,Hcy also promoted the upregulation of MPO level even at the 10 μmol/L in the cultured whole blood. The intracellular ROS, particular the $OH^{\cdot}$ radicals, play extremely important role in the Hcy-induced MCP-1 and IL-8 production**. CONCLUSION**: Increased Hcy level in plasma (hyperhomocysteinemia) induced MCP-1 and IL-8 secretion in cultured human whole blood, especially in monocytes via oxidative stress mechanism**.**

INTRODUCTION

Hcy was a sulfur-containing amino acid formed during the metabolism of methionine. Hyperhomocysteinemia, the elevated plasma Hcy levels, was defined as an independent risk factor for coronary heart disease and thrombosis[1,2]. Whether hyperhomocysteinemia is causal or merely a marker for cardiovascular disease is subject of investigation.

Chemokines belong to a super family of structure-related small chemotactic cytokines involved in leukocyte trafficking. Monocyte chemoattractant protein-1 (MCP-1), a prototype of CC chemokine[3], and interleukin-8 (IL-8), a prototype of CXC chemokine[4], were crucial in converting rolling monocytes to firm adhesion on endothelial monolayers and played pivotal role in the systemic thrombosis formation[5-9]. This strongly implied that increased plasma MCP-1 and IL-8 might promote the progression of atherosclerotic and thrombotic lesions.

Hcy could promote the expression and secretion of MCP-1 and IL-8 from cultured human aortic endothelial cells[10,11], MCP-1 from a human smooth muscle cell line[12]. All the studies suggested that Hcy could

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promote the local plaque development by increasing the MCP-1 and IL-8 level locally.

The current study was to investigate whether increased plasma Hcy level could promote the MCP-1 and IL-8 level in cultured whole blood. If true, it would indicate directly that hyper-homocysteinemia was not only the mark but also the vital causation of vascular disease development.

MATERIALS AND METHODS

Human whole blood culture The investigation conformed to the principles outlined in the Declaration of Helsinki. The human whole blood cultures were slightly modified from our previous studies[13,14]. Briefly, blood from healthy donors was drawn into heparinized syringes. Part of whole blood was then placed on a rotator and incubated in an atmosphere containing 5 % CO_2 at 37 ºC. The other portion of the whole blood was separated into primary cultures of monocytes, lymphocytes, and neutrophils. To prepare these, we used the density gradient from Nycoprep 1.077 (Life Technologies, Grand Island, NY). Blood was layered over the gradient, and the cells were separated by centrifugation. Peripheral blood mononuclear cells (PBMC) and neutrophils were obtained respectively. Monocytes were then enriched from PBMC by adherence to serum-coated culture flasks for 2 h. Adherent cells (monocytes) and suspended cells (lymphocytes) were collected. Isolated cells were plated in flat bottom of 96-well culture plates at a density of 5×10^5 (monocytes), 1.5×10^6 (lymphocytes), and 5×10^6 (neutrophils). Cell viability was evaluated by Trypan blue exclusion. Only cell preparations with a 95 % or greater viability were used.

Measurement of MCP-1 and IL-8 protein secretion Human whole blood was treated with Hcy for indicated times and/or preincubated for 60 min with antioxidants including De_2SO, catalase (CAT) 250 kU/L, catalase-polyethylene glycol (PEG-CAT)250 kU/L, superoxide dismutase (SOD) 500 kU/L or superoxide dismutase-polyethylene glycol (PEG-SOD) 500 kU/L or other pharmacological reagents. The plasma was harvested and transferred to other polypropylene tubes and stored at -30 ºC for not more than 1 week before measurement of chemokines and cytokines. TNF-α, IL-1β, MCP-1, and IL-8 protein concentrations in the plasma were determined by ELISA (R&D Systems Inc, Minneapolis, MN).

Measurement of LDH release Cultured human whole blood was treated with Hcy 10-1000 μmol/L for 32 h. Cell suspensions were collected for determination of LDH. LDH activity was measured by spectrophotometric enzyme activity method and expressed as units per liter medium.

Chemicals *L*-Hcy, *L*-cysteine, and *L*-methionine, were purchased from Sigma Co (St Louis, MO). RPMI-1640 was purchased from Gibco Laboratories (Grand Island, NY). Other chemicals were purchased from the Chinese Chemical Co (Beijing, China).

Statistical methods Results were expressed as mean±SD. The number of samples used for each group was presented in the figure legends. The data were analyzed using one-way ANOVA and further analyzed using the Student-Newmen-Keuls test for multiple comparisons within treatment groups or *t*-test (unpaired test with Welch's correction) for comparison between two groups with non-normal distribution. $P<0.05$ was considered significant between treatment groups.

RESULTS

Effect of Hcy on MCP-1 and IL-8 levels from cultured human whole blood Hcy induced the secretion of MCP-1 and IL-8 above basal levels within 4 h, and the chemokine levels continued to increase throughout the remainder of the time course (Fig 1A and 1B). When human whole blood was treated with increasing concentrations of Hcy (10-1000 μmol/L) for 32 h, Hcy as little as 10 μmol/L increased the secretion of MCP-1 production which was higher than that at Hcy 100 μmol/L (Fig 2A). However, the difference between Hcy 1000 μmol/L group and control group was not significant. The concentration-dependent effect of Hcy on IL-8 protein production was slightly different (Fig 2B) since it reached the maximal level at Hcy 100 μmol/L and there was no reduction at Hcy 1000 μmol/L.

Effect of Hcy on secretion of MPO in cultured human whole blood Hcy 100 μmol/L induced enhancement of MPO secretion above basal levels since 16 h later (Fig 3A). When human whole blood was treated with increasing concentrations of Hcy (10-1000 μmol/L) for 32 h, Hcy as little as 10 μmol/L was able to enhance the level of MPO (Fig 3B).

Effect of Hcy on LDH release from cultured human whole blood To test whether the concentrations of Hcy used in the experiment have any toxic effect to the cultured whole blood cells, the LDH concentration of plasma from the whole blood incubated

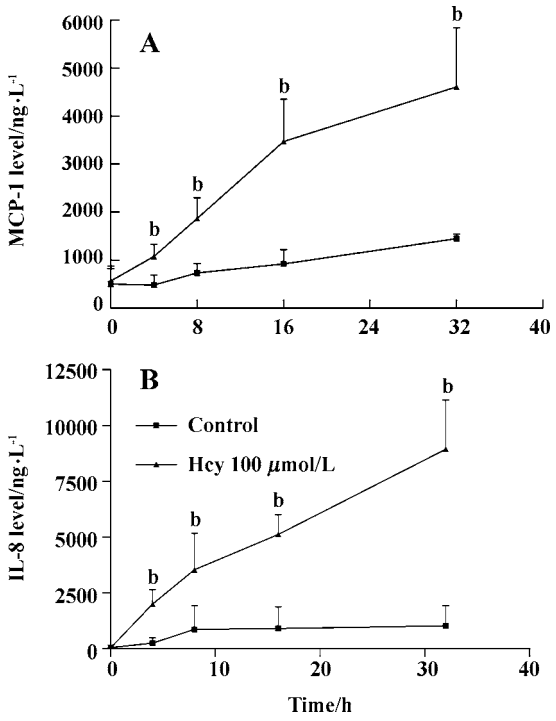

Fig 1. Time course of Hcy 100 μmol/L on secretion of MCP-1 (A) and IL-8 (B) in cultured human whole blood. *n*=5 independent experiments. Mean±SD. $^{b}P<0.05$ *vs* corresponding untreated groups.

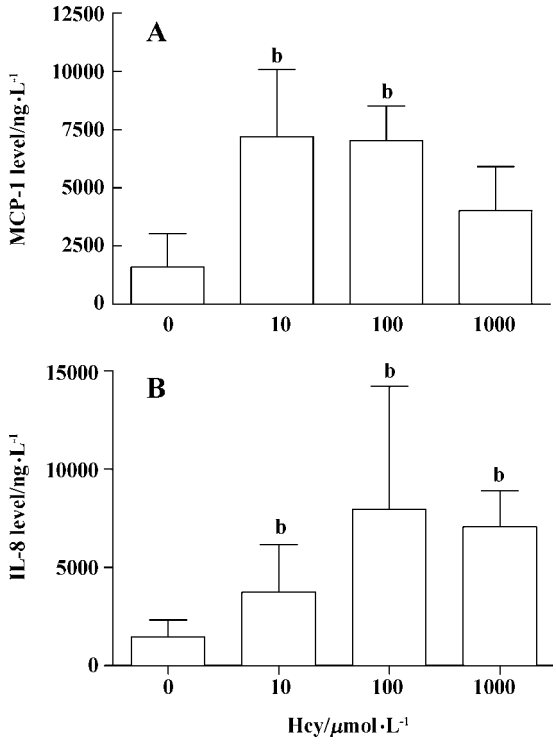

Fig 2. Effect of Hcy on secretion of MCP-1 (A) and IL-8 (B) in cultured human whole blood. *n*=5 independent experiments. Mean±SD. $^{b}P<0.05$ *vs* corresponding untreated groups.

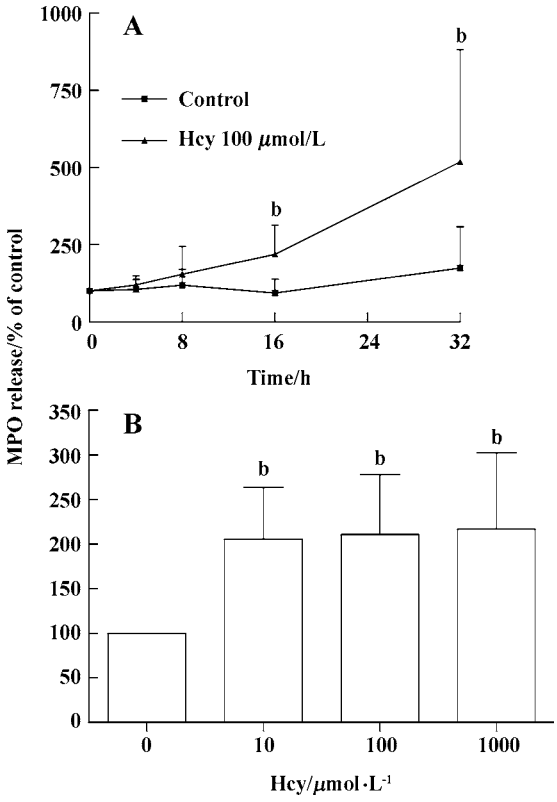

Fig 3. Effect of Hcy on the secretion of MPO in cultured human whole blood. (A) Whole blood was incubated with Hcy 100 μmol/L for different periods; (B) Whole blood was treated with different concentrations of Hcy (10-1000 μmol/L) for 32 h. *n*=5 independent experiments. Mean±SD. $^{b}P<0.05$ *vs* corresponding untreated groups.

with various concentrations of Hcy for 32 h was tested. Compared with the control, the Hcy concentrations used in our study have no significant toxic effect to cultured blood cells (Fig 4).

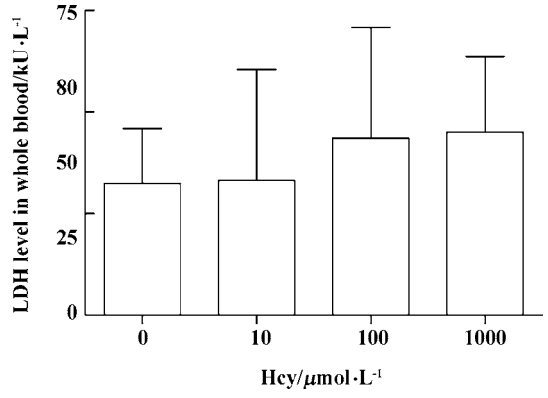

Fig 4. Effect of Hcy on LDH release from cultured human whole blood. Whole blood was incubated with various concentrations of Hcy for 32 h. *n*=5 independent experiments. Mean±SD. $^{b}P<0.05$ *vs* corresponding untreated groups.

Major cellular source of Hcy-induced secretion of MCP-1 and IL-8 from human whole blood After the isolated monocytes, lymphocytes, and neutrophils were treated with Hcy 100 μmol/L for 32 h, respectively, MCP-1 and IL-8 secretion were increased only in cultured monocytes. Hcy did not induce MCP-1 and IL-8 secretion from lymphocytes or neutrophils. These data suggested that the monocytes were principally responsible for the Hcy-induced MCP-1 and IL-8 secretion in cultured human whole blood (Fig 5).

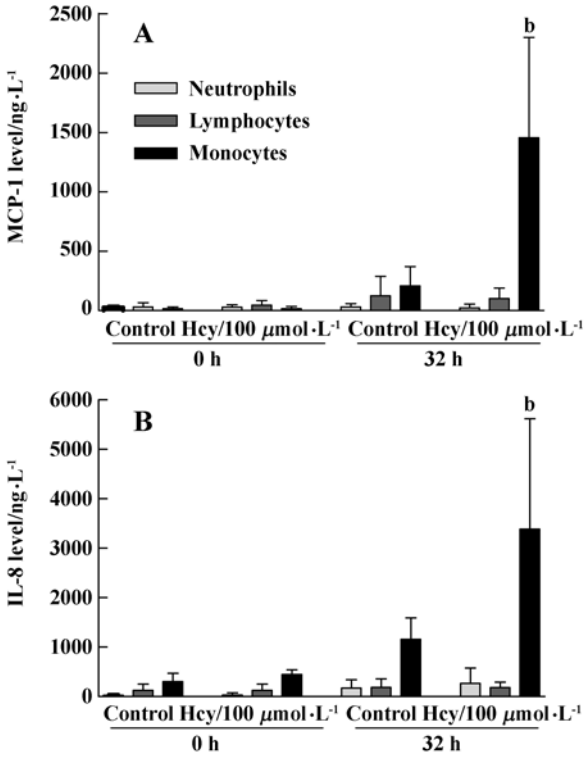

Fig 5. Effect of Hcy on the secretion of MCP-1 and IL-8 in different blood cells. The levels of MCP-1 (A) and IL-8 (B) in cultured human monocytes, lymphocytes, and neutrophils were measured by ELISA assays after incubation with Hcy 100 μmol/L for 32 h. *n*=five independent experiments. Mean±SD. $^{b}P<0.05$ *vs* corresponding untreated groups.

Role of ROS in Hcy-induced secretion of MCP-1 and IL-8 from human whole blood and monocytes To test the role of ROS in Hcy-induced chemokine secretion, whole blood and monocytes were pretreated for 60 min with antioxidant reagent 1/1000 De_2SO and 1/100 De_2SO (a scavenger of $OH^{\bullet}$), respectively. The cultured whole blood was then stimulated with Hcy 100 μmol/L for 32 h. De_2SO 1/100 significantly inhibited Hcy-induced secretion of both MCP-1 and IL-8 in whole blood (Fig 6A and 6B). In addition, whole blood was pretreated with antioxidant such as scavengers of H_2O_2, CAT and polyethylene glycol-CAT (PEG-CAT) and scavengers of $O_2^{-\bullet}$, SOD and polyethylene glycol-SOD (PEG-SOD) for 60 min, respectively. The cultured whole blood was then stimulated with Hcy 100 μmol/L for 32 h. PEG-SOD and PEG-CAT significantly inhibited Hcy-induced secretion of MCP-1 and IL-8 in human whole blood (Fig 7A and 7B). However, SOD and CAT had no such effects. Compared with SOD and CAT, polyethylene glycol which was conjugated with SOD and CAT increased enzyme ability into intracellular part in cultured cell[15]. The data suggested that Hcy-induced ROS were produced intracellularly.

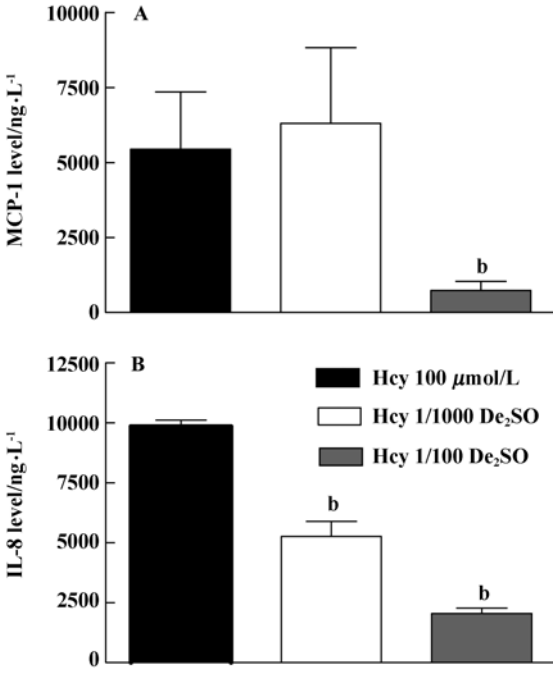

Fig 6. Inhibitory effect of De_2SO on Hcy-induced secretion of MCP-1 (A) and IL-8 (B) in cultured human whole blood. Whole blood were pretreated with 1/1000 or 1/100 De_2SO for 60 min and then stimulated by Hcy 100 μmol/L for 32 h. *n*=five independent experiments. Mean±SD. $^{b}P<0.05$ *vs* Hcy-treated groups.

DISCUSSION

The major findings of this study were that increased Hcy level in the cultured whole blood led enhancement of MCP-1 and IL-8 level. The monocytes were principally responsible for the Hcy-induced MCP-1 and IL-8 secretion in cultured human whole blood. The intracellular ROS played critical role in the Hcy-induced MCP-1 and IL-8 production. Moreover,

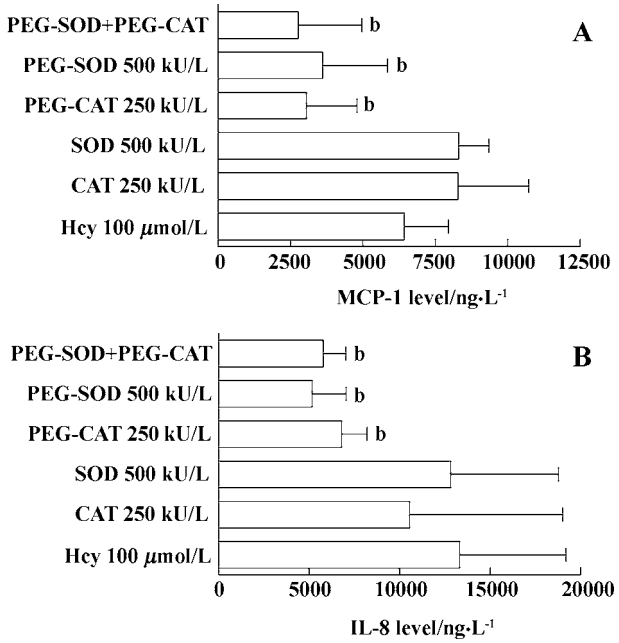

Fig 7. Inhibitory effect of antioxidants on Hcy-induced secretion of MCP-1 (A) and IL-8 (B) in cultured human whole blood. Whole blood were pretreated with antioxidants, CAT (250 kU/L), SOD (500 kU/L), PEG-CAT (250 kU/L) or PEG-SOD (500 kU/L) for 60 min and then stimulated by Hcy 100 μmol/L for 32 h, respectively. *n*=five independent experiments. Mean±SD. $^{b}P<0.05$ *vs* Hcy-treated groups.

elevated Hcy level in plasma also promoted the MPO release in the cultured whole blood.

Increased plasma MCP-1 and IL-8 levels played the pivotal role in the progression of vascular diseases and thrombosis[6-9,16,17]. Our findings provided the direct evidence to answer the question whether hyperhomocysteinemia was only cardiovascular diseases marker or the vital mediator.

Previous investigations showed that the mild hyperhomocysteinemia (10-300 μmol/L) was obviously related with increased vascular disorders[18-25]. To clarify whether the Hcy-mediated the MCP-1 and IL-8 level at such level, the concentrations that we used at current study focused within such range. Interestingly, compared with IL-8, MCP-1 production was enhanced significantly and reached the maximum level even Hcy concentration was as low as 10 μmol/L. The data suggested that the Hcy-mediated MCP-1 synthesis was much more sensitive than IL-8. Considering the Hcy levels in the most of hyperhomocysteinemia patients' plasma was in the mild range (10-30 μmol/L), our data suggested that MCP-1 might be responsible for more important pathogenesis role than IL-8 in the most of hyperhomocysteinemia patients.

The major blood cells include the monocytes, lymphocytes, and neutrophils. To find the cellular resource of MCP-1 and IL-8 synthesis in whole blood under Hcy action, such blood cells were isolated and treated with Hcy respectively. Our data demonstrated that only the monocyte-derived MCP-1 and IL-8 secretion were increased after stimulation with Hcy. It showed that the monocytes were as major cellular source to Hcy-induced MCP-1 and IL-8 synthesis in cultured whole blood. The data was similar with the previously study that the mono/macrophages functioned as major resource cells in the production of MCP-1 and IL-8 in local plaque[26-29].

Our and other previous studies showed that the mechanisms of Hcy in the atherosclerosis promotion was linked with oxidant stress and Hcy promoted the formation of ROS in human monocytes[30-33]. When Hcy was added to plasma, Hcy might undergo autooxidation that was accompanied by the production of ROS[34]. ROS could promote atherosclerosis progression by inducing vascular dysfunction and the augment of chemokine secretion[35,36]. To further define the role of ROS in the Hcy-induced chemokine production in cultured whole blood, we observed the influence of antioxidants on the Hcy-induced chemokine response. Our results indicated that Hcy-induced MCP-1 and IL-8 in cultured human whole blood were significantly inhibited by the anti-oxidants De_2SO, PEG-SOD, and PEG-CAT. Our data also showed that SOD and CAT could not inhibit the secretion of MCP-1 or IL-8 induced by Hcy. SOD and CAT are membrane-impermeable enzymes. However, conjugation of PEG to SOD and CAT increased the access of the active enzymes to intracellular compartments[15]. These data suggested that the formation of ROS played vital role in the Hcy-induced MCP-1 and IL-8 synthesis and these ROS were produced intracellularly. Moreover, we found that the Hcy-induced MCP-1 and IL-8 production were completely depressed by pretreatment with De_2SO, a scavenger of $OH^{\cdot}$ radicals, but only partly attenuated by PEG-SOD or PEG-CAT, scavengers of $O_2^{\cdot-}$ or H_2O_2. It suggested that although all the three types of ROS including $O_2^{\cdot-}$, $OH^{\cdot}$, and H_2O_2 were involved, the $OH^{\cdot}$ radicals might play the more important role in Hcy-induced secretion of MCP-1 and IL-8 in whole blood.

Our study showed that the enhancement of Hcy level in the cultured whole blood led to the upregulation of MPO concentration. MPO was as the marker of

cardiovascular event, and also considered as the important regulator of vascular disease and deep venous thrombosis[37,38]. Thus, increased MPO induced by Hcy could be one of the mechanisms by which hyperhomocysteinemia induced the initiation and progression of vascular disease. Because IL-8 was the powerful promoter of MPO release, meanwhile, we found Hcy could not promote the release of MPO from the neutrophils directly (data no shown). Thus, the Hcy-induced IL-8 secretion from the monocytes in the cultured whole blood might play the vital role in the upregulation of MPO level. However, more studies about this issue are needed to get more decisive conclusion.

Previous work suggested that cytokines such as IL-1β and TNF-α could regulate IL-8 and MCP-1 production [39]. However, our initial study found that Hcy did not enhance the secretion of TNF-α and IL-1β in cultured human whole blood (data not shown). It suggested that IL-1β and TNF-α were not involved in Hcy–induced expression of MCP-1 and IL-8 in cultured human whole blood. Therefore, the mechanism of Hcy-induced MCP-1 and IL-8 secretion was different from that of IL-1β and TNF-α.

Although as an *ex vivo* model, cultured whole blood may have more considerable relevance with respect to circulation level than isolated cultured cells, additional clinic investigation about hyperhomocysteinemia patients are still needed before the final conclusion can be addressed.

In conclusion, Hcy induced MCP-1 and IL-8 secretion in cultured human whole blood, especially in monocytes via oxidative stress mechanism. These results had several clinical implications. First, the experiment was performed at the cultured whole blood, not in the single isolated cells. Compared with the previous studies, it was more helpful to understand the influence of hyperhomo-cysteinemia. For example, we found Hcy could trigger MCP-1 and IL-8 production even at 10 μmol/L. This finding was more direct to answer the question whether Hcy enhancement (hyperhomocysteinemia) in the plasma was the marker, or the important causation of vascular diseases. Second, the upregulation of MPO revealed the possible new mechanism in Hcy-induced progression of cardiovascular diseases. Finally, the intracellular ROS, particular the $OH^{\bullet}$ radicals, played extremely important role in the Hcy-induced MCP-1 and IL-8 production. It suggested the anti-oxidative therapy would be considered to treat the hyperhomocysteinemia-related diseases.

REFERENCES

1 Malinow MR, Nieto FJ, Szklo M, Chambless LE, Bond G. Carotid artery intimal-medial wall thickening and plasma homocyst(e)ine in asymptomatic adults. The atherosclerosis risk in communities study. Circulation 1993; 87: 1107-13.

2 Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, *et al*. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 1991; 324: 1149-55.

3 Robinson EA, Yoshimura T, Leonard EJ, Tanaka S, Griffen PR, Shabanowitz J, *et al*. Complete amino acid sequence of a human monocyte chemoattractant, a putative mediator of cellular immune reaction. Proc Natl Acad Sci USA 1989; 86: 1850-4.

4 Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that attracts neutrophils. J Clin Invest 1989; 84: 1045-9.

5 Reape TJ, Groot P. Chemokines and atherosclerosis. Atherosclerosis 1999; 147: 213-6.

6 de Lemos JA, Morrow DA, Sabatine MS, Murphy SA, Gibson CM, Antman EM, *et al*. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation 2003; 107: 690-5.

7 Romuk E, Skrzep-Poloczek B, Wojciechowska C, Tomasik A, Birkner E, Wodniecki J, *et al*. Selectin-P and interleukin-8 plasma levels in coronary heart disease patients. Eur J Clin Invest 2002; 32: 657-61.

8 Van Aken BE, den Heijer M, Bos GM, van Deventer SJ, Reitsma PH. Recurrent venous thrombosis and markers of inflammation. Thromb Haemost 2000; 83: 536-9.

9 Van Aken BE, Reitsma PH, Rosendaal FR. Interleukin 8 and venous thrombosis: evidence for a role of inflammation in thrombosis. Br J Haematol 2002; 116: 173-7.

10 Poddar R, Sivasubramanian N, DiBello PM, Robinson K, Jacobsen DW. Homocysteine induces expression and secretion of monocyte chemoattractant protein-1 and interleukin -8 in human aortic endothelial cells. Circulation 2001; 103: 2717-30.

11 Sung FL, Slow YL, Wang G, Lynn EG, OK. Homocysteine stimulates the expression of monocyte chemoattractant protein-1 in endothelial cells leading to enhanced monocyte chemotaxis. Mol Cell Biochem 2001; 216: 121-8.

12 Wang G, Siow YL, O K. Homocysteine stimulates nuclear factor kappaB activity and monocyte chemoattractant protein-1 expression in vascular smooth-muscle cells: a possible role for protein kinase C. Biochem J 2000; 352: 817-26.

13. DeForge LE, Kenney JS, Jones ML, Warren JS, Remick DG. Biphasic production of IL-8 in lipopolysaccharide (LPS)-stimulated human whole blood. Separation of LPS- and cytokine-stimulated components using anti-tumor necrosis factor and anti-IL-1 antibodies. J Immunol 1992; 148: 2133-41.

14 DeForge LE, Fantone JC, Kenney JS, Remick DG. Oxygen radical scavengers selectively inhibit interleukin 8 produc-

tion in human whole blood. J Clin Invest 1992; 90: 2123-9.
15 Beckman JS, Minor RL Jr, White CW, Repine JE, Rosen GM, Freeman BA. Superoxide dismutase and catalase conjugated to polyethylene glycol increases endothelial enzyme activity and oxidant resistance. J Biol Chem 1988; 263: 6884-92.
16 Wang G, Woo CW, Sung FL, Siow YL, O K. Increased monocyte adhesion to aortic endothelium in rats with hyperhomocysteinemia: role of chemokine and adhesion molecules. Arterioscler Thromb Vasc Biol 2002; 22: 1777-83.
17 Gerszten RE, Garcia-Zepeda EA, Lim YC, Yoshida M, Ding HA, Gimbrone MA Jr, *et al*. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature 1999; 398: 718-23.
18 Mudd SH, Skovby FS, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, *et al*. The natural history of homocystinuria due to cystathione β-synthase deficiency. Am J Hum Genet 1985; 37: 1-31.
19 Selhub J, D'Angelo A. Hyperhomocysteinemia and thrombosis: acquired conditions. Thromb Haemost 1997; 78: 527-31.
20 Woo KS, Chook P, Lolin YI, Cheung AS, Chan LT, Sun YY, *et al*. Homocyst(e)inemia is a risk factor arterial endothelial dysfunction in human. Circulation 1997; 96: 2542-4.
21 Haynes WG. Hyperhomocysteinemia, vascular function and atherosclerosis: effects of vitamins. Cardiovasc Drugs Ther 2002; 16: 391-9.
22 Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, *et al*. A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. J Am Med Assoc 1992; 268: 877-81.
23 Harker LA, Slichter SJ, Scott CR, Ross R. Homocysteinemia: vascular injury and aterial thrombosis. N Engl J Med 1974; 291: 537-43.
24 Refsum H, Ueland PM. Recent data are not in conflict with homocysteine as a cardiovascular risk factor. Curr Opin Lipidol 1998; 9: 533-9.
25 Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997; 337: 230-6.
26 Nelken NA, Coughlin SR, Gordon D, Wilcox JN. Monocyte chemoattractant protein-1 in human atheromatous plaques. J Clin Invest 1991; 88: 1121-7.
27 Ylä-Herttuala S, Lipton BA, Rosenfeld ME, Särkioja T, Yoshimura T, Leonard EJ, *et al*. Expression of monocyte chemoattractant protein-1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. Proc Natl Acad Sci USA 1991; 88: 5252-6.
28 Takeya M, Yoshimura T, Leonard EJ, Takahashi K. Detection of monocyte chemoattractant protein-1 in human atherosclerotic lesions by an anti-monocyte chemoattractant protein-1 monoclonal antibody. Human Pathol 1993; 24: 539-43.
29 Apostolopoulos J, Davenport P, Tipping PG. Interleukin-8 production by macrophages from atheromatous plaques. Arterioscler Thromb Vasc Biol 1996; 16: 1007-12.
30 Zhang Q, Zeng X, Guo J, Wang X. Effects of homocysteine on murine splenic B lymphocyte proliferation and its signal transduction mechanism. Cardiovasc Res 2001; 52: 328-36.
31 Zhang Q, Zeng X, Guo J, Wang X. Oxidant stress mechanism of homocysteine potentiating Con A-induced proliferation in murine splenic T lymphocytes. Cardiovasc Res 2002; 53: 1035-42.
32 Weiss N, Heydrick S, Zhang YY, Bierl C, Cap A, Loscalzo J. Cellular redox state and endothelial dysfunction in mildly hyperhomocysteinemic cystathionine beta-synthase-deficient mice. Arterioscler Thromb Vasc Biol 2002; 22: 34-41.
33 Zeng X, Dai J, Remick DG, Wang X. Homocysteine mediated expression and secretion of monocyte chemoattractant protein-1 and interleukin-8 in human monocytes. Circ Res 2003; 93: 311-20.
34 Heinecke JW, Rosen H, Suzuki LA, Chait A. The role of sulfur-containing amino acids in superoxide production and modification of low-density lipoprotein by arterial smooth muscle cells. J Biol Chem 1987; 262: 10098-103.
34 Brandes RP, Viedt C, Nguyen K, Beer S, Kreuzer J, Busse R, *et al*. Thrombin-induced MCP-1 expression involves activation of the p22phox-containing NADPH oxidase in human vascular smooth muscle cells. Thromb Haemost 2001; 85: 1104-10.
35 Roebuck KA. Oxidant stress regulation of IL-8 and ICAM-1 gene expression: differential activation and binding of the transcription factors AP-1 and NF-kappaB (Review). Int J Mol Med 1999; 4: 223-30.
36 Baldus S, Heeschen C, Meinertz T, Zeiher AM, Eiserich JP, Munzel T, *et al*. CAPTURE Investigators: myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. Circulation 2003; 23: 1440-5.
37 Re G, Lanzarini C, Vaona I, Pazzaglia M, Palareti G, Bassein L, *et al*. Systemically circulating oxidative species in human deep venous thrombosis. Eur J Emerg Med 1998; 5: 9-12.
38 Kasahara T, Mukaida N, Yamashita K, Yagisawa H, Akahoshi T, Matsushima K. IL-1 and TNF-alpha induction of IL-8 and monocyte chemotactic and activating factor (MCAF) mRNA expression in a human astrocytoma cell line. Immunology 1999; 74: 60-7.