Arterial injury mechanism of homocysteine after PCI in patients with premature coronary heart disease

Mei Liu

The First Hospital of Hebei Medical University

Ya-Juan Yin

The First Hospital of Hebei Medical University

Ming qi Zheng

The First Hospital of Hebei Medical University

Xiao-Cui Shi

The First Hospital of Hebei Medical University

Da Liu

The First Hospital of Hebei Medical University

Dong-Yue Liu

The First Hospital of Hebei Medical University

Gang Liu (✉ cardio2004@163.com)

The First Hospital of Hebei Medical University

Mei Wei

The First Hospital of Hebei Medical University

Research Article

Keywords: Premature coronary disease, Risk factors, Homocysteine, Atorvastatin

Posted Date: February 7th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1287891/v1>

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Abstract

Background: One cause of mortality among young people in modern societies is coronary heart disease (CHD). Numerous studies have demonstrated an association between major adverse cardiac events (MACEs) and percutaneous coronary intervention (PCI) in patients with premature CHD, but the risk factors for MACEs after PCI are poorly understood. This study investigated the risk factors for premature CHD after PCI and compared the long-term prognosis of patients with premature CHD with or without MACEs after PCI. Homocysteine was found to be an independent risk factor for patients with early CHD. Previous animal studies have demonstrated that atorvastatin inhibits homocysteine-induced inflammation and that atorvastatin has preventive and therapeutic effects in patients with early CHD.

Methods: A total of 1408 patients who underwent PCI were enrolled and divided into a MACE group and a non-MACE group according to the recurrence of cardiovascular events. Basic information and follow-up data of the patients were recorded, and risk factors affecting prognosis were analysed. Western blotting and reverse transcription polymerase chain reaction were used to identify that atorvastatin inhibited the expression of NF- κ B, IL-1 β , IL-6, and CRP inflammatory factors induced by homocysteine, thereby inhibiting the occurrence and development of atherosclerosis.

Results: Univariate Cox proportional risk models showed that the homocysteine level is a risk factor for recurrent MACEs. Multivariate Cox regression analysis also suggested that homocysteine is an independent risk factor for MACEs. The receiver operating characteristic curve showed that Hcy was a strong factor for predicting the occurrence of MACEs in patients with premature CHD. Atorvastatin was found to inhibit the expression of NF- κ B, IL-1 β , IL-6, and CRP inflammatory factors induced by homocysteine and delay the occurrence of reactive oxygen species.

Conclusions: Homocysteine is an independent risk factor for MACEs in patients with premature CHD after PCI. Atorvastatin can effectively antagonize the expression of NF- κ B, IL-1 β , IL-6, CRP and other inflammatory factors induced by homocysteine.

Introduction

Coronary heart disease (CHD) has one of the highest morbidity and mortality rates in both developed and developing countries. Although acute myocardial infarction mainly occurs in the elderly population, the average age of individuals suffering from CHD is gradually becoming younger. CHD in a younger population is known as premature coronary heart disease (PCHD). Approximately 4~10% of patients with acute myocardial infarction are aged ≤ 45 years[1]. PCHD patients are defined as patients aged ≤ 55 years[2]. At present, the prevalence and incidence of CHD are increasing among younger Chinese people[3]. Thrombolysis and revascularization (percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG)) are the main treatment methods for young patients with CHD. However, a significant proportion of young patients subsequently experience a major adverse cardiovascular event (MACE), including all-cause death, nonfatal myocardial infarction, PCI and CABG revascularization, or

stroke. MACEs affect not only the patient's physical health but also their mental health. Therefore, it is important to effectively prevent the occurrence and recurrence of CHD in younger people. Compared with studies of elderly CHD patients, studies on the prevention and long-term prognosis of those with PCHD are limited. This study analysed the risk factors for MACEs in patients with PCHD after PCI and provide evidence for the treatment and prevention of PCHD.

Materials And Methods

Study design and population

A total of 1624 patients were enrolled and evaluated between May 2017 and October 2020 at the First Affiliated Hospital of Hebei Medical University. The inclusion criteria were as follows: (1) males aged ≤ 45 years and females aged ≤ 55 years; (2) patients with symptoms of acute coronary syndrome at admission, including chest pain, chest tightness, palpitations and other symptoms, and indications for coronary angiography; (3) patients diagnosed with unstable new angina pectoris, ST-segment elevation myocardial infarction, or non-ST-segment elevation myocardial infarction; and (4) agreed to undergo coronary angiography and sign an informed consent form. The exclusion criteria were as follows: severe cardiac insufficiency (New York Heart Association classification greater than grade III); severe cardiomyopathy; other serious systemic diseases, such as tumours and immune system diseases; and severe hepatic or renal insufficiency, such as late renal insufficiency. The total number of patients included was 1408, with a loss to follow-up rate of 13.3%.

Data collection

The clinical baseline data of patients were collected and included diagnosis, age, sex, history of hypertension, and history of diabetes. Angiography results included the number of lesions, number of stents, and total length of stents. Blood test results included total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), APOA, APOB, lipoprotein, fibrinogen, high-sensitivity C-reactive protein (CRP), random blood glucose, creatinine, uric acid, homocysteine (Hcy), and left ventricular ejection fraction (LVEF).

Follow-up

Patients were followed up by telephone every 3 months after discharge. The follow-up assessment included whether patients suffered from MACEs and medication status, mainly including the use of aspirin, clopidogrel, statins, nitrates, angiotensin-converting enzyme inhibitor (ACEI)/angiotensin receptor blocker (ARB) drugs, β -blockers and calcium antagonists. Survival time for the MACE group was defined as the time from discharge to the occurrence of a MACE. Survival time for the non-MACE group was the duration until the end of this study. Patients who were lost to follow-up were censored at the last known contact date.

Cell culture

Primary vascular smooth muscle cells (VSMCs) were isolated from rat aortas[4]. The isolated cells were maintained in low-glucose Dulbecco's modified Eagle's medium (Invitrogen) containing 10% foetal bovine serum (Gibco), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells from passages 3 to 5 were used for further experiments.

Reverse transcription polymerase chain reaction (RT–PCR)

Total RNA was extracted using TRIzol reagent (Life Technologies) following the manufacturer's instructions. To quantify the amount of mRNA, cDNA was synthesized using the M-MLV First Strand Kit (Life Technologies), and quantitative PCR was performed using SYBR Green qPCR SuperMix-UDG (Life Technologies). Relative mRNA expression was normalized to the β -actin/GAPDH levels using the $2^{-\Delta\Delta C_t}$ method. The average threshold cycle for each gene was determined from at least three independent experiments.

Western blot analysis

Lysates from cells or tissues were prepared with RIPA lysis buffer. Equal amounts of protein were separated by SDS–PAGE and electrotransferred to a PVDF membrane. The membranes were blocked with 5% nonfat dairy milk and incubated with primary antibodies against anti-IL-1 β (1:500 dilution), anti-IL-6 (1:500 dilution), anti-NF- κ B (1:1000 dilution), and anti-GAPDH (1:2000 dilution) at 4°C overnight. This was followed by incubation with IRDye800[®]-conjugated secondary antibody (1:2000 dilution) for 1 h at room temperature and subsequent scanning with an Odyssey Infrared Imaging System (LI-COR Biosciences). The integrated intensity for each detected band was determined using Odyssey Imager software. The data are presented as the mean $\pm$ standard deviation (SD) from at least three independent experiments.

Determination of reactive oxygen species (ROS)

The H2DCF-DA fluorescence labelling method was used to detect ROS generated in VSMCs. The VSMCs were incubated with 10 µM atorvastatin[5] for 24 h, followed by incubation with H2DCF-DA (10 µM) for 30 min at 37°C, and then placed in Hcy (100 µM) [6]for 40 min. The fluorescence signal was monitored by fluorescence microscopy.

Statistical analysis

The data analysis was performed using SPSS version 21.0. Patients undergoing coronary angiography were divided into the MACE group and the non-MACE group according to the occurrence of MACEs. The measurement data with a normal distribution are expressed as the mean $\pm$ SD ($X \pm SD$), and comparisons between groups were performed by *t*-test. The count data that did not conform to a normal distribution are represented by the median (interquartile range), and comparisons between groups were performed with the Kruskal–Wallis method. Categorical variables are expressed as counts and percentages and were compared using the χ^2 test or Fisher's exact test when the number of events was low. Univariate and

multivariate Cox proportional risk regression models were used to analyse the risk of MACEs. The correlations between MACE risk and predictive variables were expressed as the hazard ratio (HR) of first MACE occurrence. $P < 0.05$ was considered statistically significant. The predictive value of Hcy for MACE events was analysed by receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) was calculated. All data are expressed as the mean $\pm$ SD ($\bar{x} \pm s$), and differences between groups were analysed by one-way ANOVA, followed by Tukey's test. $P < 0.05$ was considered statistically significant.

Results

Baseline data

A total of 1408 patients were enrolled, with 146 patients developing MACEs and 1262 patients not developing MACEs during the follow-up period from 1 March 2015 to 31 December 2018. The baseline data of the patients are shown in Table 1. The median patient age was 44.54 (40.84-51.10) years; 560 women (39.80%) and 848 men (60.20%) were included. The mean age of 46.20 (43.00-53.00) years in the MACE group was significantly higher than the mean age of 44.39 (40.52-50.68) years in the non-MACE group ($P < 0.001$). Overall, 57.5% of the MACE group were women, whereas 37.7% of the non-MACE group were women. As traditional risk factors, the prevalences of hypertension history, diabetes history and family history of CHD in the MACE group were higher than those in the non-MACE group ($P < 0.05$). The number of patients diagnosed with unstable angina was higher in the MACE group than in the non-MACE group ($P = 0.038$), while the number of patients diagnosed with ST-elevation myocardial infarction was lower in the MACE group than in the non-MACE group ($P = 0.015$). The level of homocysteine in the MACE group was higher than that in the non-MACE group ($P < 0.001$). There were fewer patients taking oral β -blockers in the MACE group (62.3%) than in the non-MACE group (75%), but there were more patients using calcium antagonists in the MACE group ($P = 0.045$).

Univariate COX regression proportional risk model

The results of the risk factor analysis of CHD patients after PCI are shown in Table 2. Compared with young patients, elderly patients had a higher risk of MACEs (HR, 1.292, 95% CI, 1.029-1.381, $P < 0.001$), and women were more likely to develop MACEs than men (HR, 2.149, 95% CI, 1.547-2.985, $P < 0.001$). Among the traditional risk factors, patients with a history of diabetes (HR, 1.509, 95% CI, 1.077-2.114, $P = 0.017$) and a family history of CHD (HR, 1.566, 95% CI, 1.093-2.243, $P = 0.014$) were more likely to develop MACEs again. Patients with high levels of homocysteine were more likely to develop MACEs again (HR, 1.012, 95% CI, 1.006-1.018, $P < 0.001$). Postoperative use of β -blockers was a protective factor (HR, 0.581, 95% CI, 0.416-0.812, $P = 0.001$), while the use of calcium antagonists was a risk factor (HR, 1.489, 95% CI, 1.026-2.163, $P = 0.036$).

Multivariate COX regression proportional risk model

The multivariate Cox proportional risk model is shown in Table 3. Female sex (HR, 5.546, 95% CI, 1.303-4.975, $P=0.006$), history of diabetes (HR, 1.827, 95% CI, 1.153-2.895, $P=0.010$), family history of CHD (HR, 1.458, 95% CI, 1.002-2.121, $P=0.049$) and homocysteine (HR, 1.018, 95% CI, 1.011-1.025, $P<0.001$) were independent risk factors for MACEs after PCI in patients with premature CHD. The use of β -blockers was a protective factor (HR, 0.626, 95% CI, 0.434-0.904, $P=0.012$). Over time, the MACE survival rate of these patients gradually decreased (Figure 1A), while the cumulative risk factors gradually increased (Figure 1B).

The predictive value of Hcy for MACEs

The ROC curve showed that Hcy was a strong predictive factor for the occurrence of MACEs in patients with premature CHD (Figure 2). The AUC was 0.713 (95% CI: 0.679-0.748, $P<0.001$), the sensitivity was 82.9%, and the specificity was 53.6%. The optimal Hcy threshold was 11.45 $\mu\text{mol/L}$.

Effect of atorvastatin on CRP expression induced by Hcy in VSMCs

As shown in Figure 3, the expression of CRP mRNA and protein increased after incubating VSMCs with 100 μM homocysteine for 12 h ($P<0.05$). CRP expression was significantly inhibited by the administration of 10 μM atorvastatin 24 h prior to homocysteine stimulation. In addition, atorvastatin alone stimulated VSMCs and had no effect on the basal CRP levels.

Effects of atorvastatin on homocysteine-induced ROS expression

As shown in Figure 4, a small amount of fluorescence was observed in the VSMCs of the control group, indicating the generation of basal levels of ROS. After incubating the cells in 100 μM homocysteine for 40 min, the fluorescence was significantly enhanced compared with that of the control group ($P<0.05$). After incubation with 10 μM atorvastatin for 24 h, the cellular fluorescence was significantly decreased, indicating that ROS production was significantly decreased ($P<0.05$).

Effect of atorvastatin on NF- κ B expression induced by homocysteine

As shown in Figure 5, Western blotting was used to detect NF- κ B expression stimulated by homocysteine. As shown in the figure, 100 μM homocysteine promoted NF- κ B expression (vs. control, $P<0.05$), while 10 μM atorvastatin attenuated the effect of homocysteine on NF- κ B expression (vs. Hcy + 10 μM atorvastatin).

Effect of atorvastatin on the expression of IL-1 β and IL-6 induced by homocysteine

As shown in Figure 6, Western blotting and RT-PCR were used to detect homocysteine-induced IL-1 β and IL-6 expression. VSMCs were pretreated with 10 μM atorvastatin for 24 h and then incubated with 100 μM homocysteine for 12 h. The homocysteine treatment promoted the protein and mRNA expression of IL-1 β and IL-6 in cells (vs. control, $P<0.05$). However, 10 μM atorvastatin significantly inhibited the expression of IL-1 β and IL-6 induced by homocysteine (vs. Hcy + 10 μM atorvastatin, $P<0.05$).

Discussion

An increasing number of studies have focused on PCHD, but the risk factors and clinical characteristics of patients with PCHD are still poorly understood, and the optimal treatment of these patients remains unclear. In this study, risk factors for PCHD were analysed to provide evidence for preventing PCHD and improving the clinical prognosis.

In studies conducted in China and abroad, the proportion of men with PCHD has been found to be higher than that of women. Among PCHD patients, the proportion of men is approximately 79~95%[7]. According to Chan *et al.*, of PCHD patients younger than 45 years, approximately 90% are men[8]. This suggests that young men are more likely to have CHD than women, possibly because men have more risk factors. In addition, oestrogen may play a protective role in women[9]. In this study, the Cox regression proportional risk model showed that female sex was an independent risk factor for MACEs, which were more common among women than among men. In one study, women accounted for a larger proportion (32% vs. 41.5%) of nonblocking premature coronary artery disease patients. Compared with young men, young women also had a higher risk of adverse cardiovascular events and poorer prognosis in patients with obstructive (> 50% coronary artery stenosis) myocardial infarction[10].

Hcy is a nonprotein-forming amino acid and a direct metabolic precursor of methionine. The normal range of plasma Hcy levels is 4-15 $\mu\text{mol/L}$, and when the plasma level exceeds 15 $\mu\text{mol/L}$, it is referred to as hyperhomocysteinemia[11]. In 1969, McCully *et al.* first proposed the concept that hyperhomocysteinemia is an independent risk factor for cardiovascular disease[12]. A number of studies have shown that Hcy can promote the formation of coronary atherosclerosis through a variety of mechanisms[13], but the specific injury mechanism remains unclear. Relevant studies have shown that homocysteine can lead to endothelial dysfunction and trigger an inflammatory response, leading the cascade of atherosclerotic lesions[14]. In addition, homocysteine induces oxidative stress and increases the production of NADPH oxidase, which in turn increases the production of superoxides such as ROS. Experiments have shown that superoxide production is induced in in vitro aortic endothelial cell cultures at the same Hcy concentration as the physiological level (1 mM)[15]. In the current study, Hcy was confirmed to be an independent risk factor for MACEs in patients with PCHD after PCI, which is basically consistent with the conclusions of previous studies. Moreover, Hcy induced the expression of NF- κ B, IL-1 β , IL-6, CRP and other inflammatory factors. Related experimental studies have confirmed that Hcy mediates CRP production through the nMDA-ROS-ERK1/2/P38-NF- κ B signalling pathway[16]. Therefore, whether improving the control of Hcy levels can reduce endothelial injury and inflammation needs to be further studied.

As mentioned above, Hcy can induce endothelial injury and an inflammatory response, promoting the occurrence and development of atherosclerosis. In most patients, taking B vitamins is an effective way to reduce serum homocysteine[17], but this intervention does not reduce the occurrence of MACEs in patients with CHD, nor does it improve long-term prognosis. One possible hypothesis for this is that B vitamins promote the occurrence of atherosclerosis; one study confirmed that patients taking B vitamins

had a higher rate of vascular restenosis after PCI[18]. In addition to causing cardiovascular damage, elevated homocysteine levels may also cause hypothyroidism[19]. Therefore, further research is needed to determine how to reduce the homocysteine level and improve the clinical prognosis.

In addition to increasing oxidative stress, high levels of Hcy can also induce inflammation, accelerating the development of atherosclerosis. Extensive laboratory and clinical data suggest that atherosclerosis is a chronic inflammatory disease. Inflammation plays an important role in the progression from initial endothelial dysfunction to plaque formation and rupture[20]. Elevated circulating Hcy levels can increase the adhesion ability of T cells and monocytes to the endothelium, and these cells can induce VSMCs to release inflammatory factors such as IL-1 β , IL-6, MCP-1 and MMP-2[21]. Therefore, Hcy can indirectly promote the inflammatory response and accelerate the occurrence and development of atherosclerosis. As mentioned above, Hcy promotes oxidative stress and ROS production in vascular smooth muscle, and many in vivo and in vitro experiments have also demonstrated that high levels of Hcy increase ROS production in the circulation[22]. As an important messenger of intracellular signal transduction, ROS mediates the production of inflammatory factors through MAPK, NF- κ B and other signalling pathways[23]. In this study, we confirmed in vitro that high levels of Hcy promote NF- κ B expression in vascular smooth muscle and increase transcription factors known to play an important role in inflammatory and immune processes. NF- κ B plays a central role in inflammation by participating in the binding of chemokines, adhesion factors and growth factors[24] and plays an important role in the inflammation and apoptosis associated with coronary atherosclerosis[25]. In addition, NF- κ B not only promotes inflammation during atherosclerosis but also affects angiogenesis, which is an important risk factor for coronary atherosclerosis.

In addition, NF- κ B is involved in the Hcy-induced expression of CRP, which is a representative marker of atherosclerosis[26]. CRP binds to LDL and is present in atherosclerotic plaques. Studies have shown that in the general population, blood CRP levels independently predict the risk of all-cause and cardiovascular mortality[27]. During inflammation, IL-1 β and IL-6 are mainly produced in the liver and positively regulate CRP production. IL-1 β expression is elevated during the development of atherosclerotic lesions, suggesting IL-1 β as a clinical target for the treatment and prevention of atherosclerosis. In addition, as a multipotent cytokine, IL-6 regulates the immune system and participates in acute and chronic inflammatory processes[28]. Studies have found that the more severe the disease in patients with CHD, the higher the inflammation index; furthermore, patients with ruptured atherosclerotic plaques have higher IL-6 levels[29]. Therefore, IL-6 might be an effective marker for predicting atherosclerotic plaques.

Statins are HMG-CoA reductase inhibitors and are currently the most widely used lipid-lowering drugs; they can stabilize atherosclerotic plaques and play an important role in preventing cardiovascular events[30]. A meta-analysis found that regardless of a patient's clinical manifestations, the active use of statins significantly reduces the recurrence rate of myocardial infarction after PCI and decreases the occurrence of MACEs within 30 days by 44%[31]. As first-line lipid-lowering drugs used in clinical practice, statins inhibit the expression of LDL receptors in the liver through competitive induction and increased LDL catabolism, thus playing a lipid-lowering role. However, recent studies have found that the effect of

statins on reducing the morbidity and mortality of cardiovascular diseases is due to not only the excellent lipid-lowering effect but also other effects, such as improving endothelial function and antithrombosis, in addition to the anti-inflammatory effect of statins[32]. One study showed that the expression of the inflammatory factors MCP-1, IL-6, and IL-8 could be detected in coronary atherosclerotic plaques, and the constitutive expression of MCP-1, IL-6, and IL-8 was significantly reduced after statin treatment. Many clinical studies suggest that the use of statins can significantly reduce the generation of circulating CRP and improve the clinical prognosis of patients with CHD. Studies have confirmed that hyperhomocysteinemia promotes the development of coronary atherosclerosis and plaque instability. It has also been shown that atorvastatin downregulates homocysteine-induced atherosclerosis and endoplasmic reticulum stress activation, inhibits the inflammatory response, and plays a role in plaque stability. In addition, relevant studies indicate that atorvastatin downregulates the expression of Hcy-induced inflammatory factors such as MMP-9 and TNF- α [33], thus playing an anti-inflammatory role. Increasing clinical data show that atherosclerosis also occurs in patients with normal or low lipid levels[34]. An increasing number of studies have confirmed that inflammation plays an important role in plaque formation. In this study, atorvastatin was found to reduce intracellular ROS production and downregulate the expression of NF- κ B, IL-1 β , IL-6 and CRP, thus playing an anti-inflammatory role and protecting VSMCs. These results are consistent with previous studies, but the protective effect of atorvastatin against cardiovascular disease needs to be further investigated.

In addition to imperceptible risk factors, such as the level of cysteine in the body, other risk factors, such as family history and diabetes history, can increase the risk of the recurrence of MACEs after PCI. Some factors are controllable and others are not. With economic development and changes in lifestyle over the past 30 years, the prevalence and incidence of hypertension, CHD and diabetes in China have been increasing annually[35], and the affected population is gradually becoming younger. The risk factors described above promote the occurrence and development of CHD. This study found a statistically significant difference in the history of diabetes between the MACE group and the non-MACE group, and multivariate Cox regression further confirmed that a history of diabetes was an independent risk factor for MACE recurrence in patients with PCHD. Generally, compared with elderly patients, PCHD patients more often have traditional risk factors, such as diabetes and a family history of CHD[36]; therefore, their risk is different from that of elderly CHD patients. In addition, as a traditional risk factor, a history of diabetes can be used to predict the likelihood of long-term recurrence events and the mortality of young patients with CHD[37]. Therefore, improving the diet and activity levels of younger people and reducing the occurrence of diabetes would significantly prevent CHD. Unlike controllable risk factors, such as diabetes history, a family history of CHD is a risk factor that cannot be prevented. In a previous study, approximately one-quarter of the population with a family history of CHD was at high risk of CHD, requiring premature intervention to prevent the occurrence of PCHD[38]. A study based on a community population in the United States showed that the prevalence rate of PCHD in young people with a positive family history of CHD was approximately 75%[39]. Other studies have shown that the prevalence of CHD in young people with a family history of CHD is twice that of elderly individuals[40], and some data even show that the prevalence is 4 times that of elderly individuals[8]. Therefore, as a strong independent

predictor of PCHD, a family history of CHD could be used as an indicator for targeted interventions to help prevent or delay the occurrence of PCHD.

β -Adrenergic receptor blockers have a strong effect on reducing myocardial oxygen consumption, and they antagonize catecholamine arrhythmia, reducing the rate of myocardial reinfarction. Clinical trials have shown that the long-term use of β -blockers can significantly reduce the mortality of patients with acute myocardial infarction and greatly improve their clinical prognosis[41]. According to current guidelines, patients with myocardial infarction should regularly take β -blockers as long as there are no contraindications[42]. The role of β -blockers in patients with myocardial infarction is well characterized. In the current study, the use of β -blockers in the MACE group was lower than that in the non-MACE group, and the multivariate Cox regression proportional risk model suggested that the use of β -blockers was a protective factor, which is consistent with previous findings and guidelines. However, a national study in 2017 showed that while β -blockers significantly reduced mortality in patients with acute myocardial infarction, they did not reduce mortality in patients with angina pectoris, and the survival benefit in patients with myocardial infarction was significant only within one year of acute myocardial infarction[43]. However, an increasing number of studies have shown that the mortality of patients with myocardial infarction continues to increase after the initiation of β -blocker use, and the survival time ranges from several months to several years after the occurrence of myocardial infarction[44]. The American Heart Association recommends β -blockers as the first-line treatment for acute myocardial infarction[42], but European guidelines recommend against β -blockers for individuals who have acute myocardial infarction with reduced cardiac function[45]. Although the current study showed that the use of β -blockers in young patients was protective against the occurrence of MACEs after PCI, there is no relevant study on whether the premature use of β -blockers in young patients reduces the occurrence of MACEs. Therefore, whether the protective effect of β -blockers found in this study is related to patient age or other drug interactions requires further clarification.

Limitations

The clinical part of the study was a single centre study. No randomized controlled study was conducted to compare the homocysteine levels in CHD patients with those in the general population. A multicentre study could be conducted to compare the homocysteine levels in patients with those in the general population. The optimal concentration stated in the experimental section was selected based on previous studies, and a concentration gradient was not set. Future studies should conduct a concentration gradient experiment.

Conclusion

Homocysteine is an independent risk factor for cardiovascular disease and can promote the development of cardiovascular injury by inducing inflammation. Atorvastatin attenuates the homocysteine damage to vascular smooth cells, thereby protecting the cardiovascular system.

Declarations

Acknowledgements

All authors declare that they have no conflicts of interest.

Authors' contributions

Liu M, Wei M and Yin Y developed the idea for the study, participated in its design and coordination, and helped draft the manuscript. Liu D, Shi X and Liu D contributed to data acquisition and interpretation. Liu G and Zheng M provided critical review and substantially revised the manuscript. All authors read and approved the final manuscript.

Funding

The study was supported by the Key Project of Hebei Science and Technology Support Plan [grant numbers 203777117D and 19277757D], the Natural Science Foundation of Hebei Province [grant numbers H2021206217 and H2019206715], and the Key Science and Technology Research Program of Hebei Provincial Health Commission [grant numbers 20210065 and 20190436].

Ethics approval and consent to participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All animal procedures conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of Hebei Medical University.

Consent for publication

Written informed consent for publication was obtained from all participants.

Competing interests

The authors declare that they have no competing interests.

Author details

¹ Department of Cardiovascular Medicine, The First Hospital of Hebei Medical University, No. 89

Donggang Road, Shijiazhuang 050031, Hebei, China.

² Heart Center, The First Hospital of Hebei Medical University, No. 89 Donggang Road, Shijiazhuang 050031, Hebei, China.

Abbreviations

CHD: Coronary heart disease; MACEs: Major adverse cardiac events; PCI: Percutaneous coronary intervention; PCHD: Premature coronary heart disease; CABG, Coronary artery bypass grafting; UA, Unstable angina pectoris; NSTEMI, Non-ST-segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; LVEF: Left ventricular ejection fraction; HDL: High-density lipoprotein; LDL, Low-density lipoprotein; VLDL, Very-low-density lipoprotein; APOA, Apolipoprotein A; APOB, Apolipoprotein B; Hs-CRP: High-sensitivity C-reactive protein; ACEI, Angiotensin-converting enzyme inhibitor; ARB, Angiotensin receptor antagonist.

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Tables

Table 1 Baseline data of all patients, the MACE group and the non-MACE group^a

Variable	Patients (n=1408)	MACE event group (n=146)	Non-MACE event group (n=1262)	P value
Age (years old)	44.54 (40.84-51.10)	46.20 (43.00-53.00)	44.39 (40.52-50.68)	0.001 ^b
Sex (female %)	560 (39.80)	84 (57.5)	476 (37.7)	0.001 ^c
History of hypertension (%)	844 (59.9)	99 (67.8)	745 (59.00)	0.041
History of diabetes (%)	396 (28.1)	53 (36.3)	343 (27.2)	0.025
History of cerebral infarction (%)	90 (6.4)	11 (7.5)	79 (6.3)	0.590
Smoking history (%)	764 (54.3)	73 (50.0)	691 (54.8)	0.293
Family history of CHD (%)	272 (19.3)	42 (28.8)	230 (18.20)	0.004
Past history of myocardial infarction (%)	97 (6.9)	10 (6.8)	87 (6.9)	1.000
History of PCI (%)	103 (7.3)	9 (6.2)	94 (7.4)	0.737
History of CABG (%)	8 (0.6)	2 (1.4)	6 (0.5)	0.198
Diagnosis				
UA (%)	879 (62.4)	103 (70.5)	776 (61.5)	0.038
NSTEMI (%)	129 (9.2)	14 (9.6)	115 (9.1)	0.879
STEMI (%)	400 (28.40)	29 (19.9)	371 (29.4)	0.015
LVEF (%)	60.19 (55.33-64.10)	60.08 (56.16-64.22)	60.20 (55.18-64.09)	0.817
Cholesterol (mmol/l)	4.72 (3.98-5.55)	4.81 (3.78-5.51)	4.71 (3.99-5.54)	0.921
HDL (mmol/l)	0.97 (0.81-1.15)	1.01 (0.84-1.18)	0.96 (0.81-1.15)	0.117
LDL (mmol/l)	3.15 (2.45-3.85)	3.18 (2.37-3.87)	3.13 (2.45-3.85)	0.832
Triglycerides (mmol/l)	1.78 (1.28-2.46)	1.63 (1.31-2.23)	1.80 (1.28-2.48)	0.370
VLDL (mmol/l)	0.49 (0.32-0.72)	0.47 (0.35-0.69)	0.49 (0.32-0.73)	0.823
APOA (g/L)	1.14 (1.01-1.30)	1.17 (1.05-1.32)	1.14 (1.01-1.30)	0.185

APOB (g/L)	1.08 (0.88-1.28)	1.050.84-1.26	1.080.88-1.28	0.526
APOA/APOB	1.070.87-1.33	1.180.90-1.37	1.070.86-1.32	0.067
Lipoprotein A (mg/dl)	22.30 (9.27-64.50)	17.907.10-57.20	24.569.50-65.20	0.066
Fibrinogen (mg/dl)	3.26 (2.85-3.76)	3.282.89-3.78	3.252.84-3.76	0.507
Hs-CRP (mg/l)	2.51 (0.92-5.86)	2.240.90-5.30	2.540.93-5.99	0.381
Random blood sugar (mmol/l)	5.39 (4.83-6.53)	5.484.76-6.65	5.384.84-6.51	0.675
Creatinine (umol/l)	68.9557.70-79.92)	65.3355.92-79.00	69.4457.99-80.11	0.137
Uric acid (umol/l)	320.23 (259.84-386.81)	306.50 (246.33-365.00)	322.84(261.75-388.62)	0.058
Homocysteine (μmol/L)	11.46 (9.08-16.00)	14.7112.10-21.80	11.068.82-15.10	0.001
Coronary angiogram				
Normal (%)	171.2	32.1	141.1	0.409
Triple vessel lesion (%)	312.26	32.08	282.28	1.000
Double vessel lesion (%)	24717.5	2718.5	22017.5	0.731
Single vessel lesion (%)	111379.00	11377.4	100079.2	0.592
Left main lesion (%)	533.8	85.55	453.6	0.249
Anterior descending branch lesion (%)	859 (61.0)	9363.7	76660.7	0.531
Rotatory branch lesion (%)	347 (24.6)	3624.7	31124.6	1.000
Lesion of right coronary artery (%)	494 (35.1)	4732.2	44735.4	0.465
Average number of scaffolds	1.42 (?-1.99)	1.47-2.12	1.42-1.98	0.358
Average length of support (mm)	32.29 (21.94-51.56)	34.0019.92-52.67	32.1522.20-51.49	0.829
Medication situation				

Aspirin (%)	1396 (99.1)	145 [99.30]	1251 [99.10]	0.642
Clopidogrel (%)	1363 (96.8)	140 [95.9]	1223 [96.9]	0.458
Statins (%)	1337 (95.0)	134 [91.8]	1203 [95.3]	0.072
Nitrates (%)	527 (37.4)	59 [40.4]	468 [37.1]	0.470
ACEI/ARB (%)	755 (53.6)	75 [51.4]	680 [53.9]	0.659
β-blockers (%)	10038 [73.7)	91 [62.3]	947 [75.0]	0.001
Calcium antagonist (%)	268 (19.0)	37 [25.3]	231 [18.3]	0.045

PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; UA, unstable angina pectoris; NSTEMI, non-ST-segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; LVEF: ejection fraction; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; APOA, apolipoprotein A; APOB, apolipoprotein A; Hs-CRP: C-reactive protein; APOA, apolipoprotein A; APOB, apolipoprotein B; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor antagonist.

a Data are presented as the median (interquartile) or number (percent).

b Kruskal–Wallis method was used.

c Chi–square test was used.

Table 2. Single-factor Cox proportional hazard model analysis of the influencing factors of MACE events

Univariate COX regression				
Variable	Pvalue	HR	95%CI	
			Bottom	Top
Age (years old)	≤0.001	1.292	1.029	1.381
Sex (female %)	≤0.001	2.149	1.547	2.985
History of hypertension (%)	0.052	1.411	0.997	1.890
History of diabetes (%)	0.017	1.509	1.077	2.114
History of cerebral infarction (%)	0.483	1.246	0.697	2.304
Smoking history (%)	0.186	0.803	0.580	1.111
Family history of CHD (%)	0.014	1.566	1.093	2.243
Past history of myocardial infarction (%)	0.281	0.928	0.488	1.765
History of PCI (%)	0.438	0.766	0.390	1.503
History of CABG (%)	0.279	2.162	0.535	8.730
Diagnosis				
UA (%)	0.073	1.385	0.970	1.978
NSTEMI (%)	0.580	1.169	0.673	2.208
STEMI (%)	0.025	0.628	0.418	0.943
LVEF (%)	0.952	1.001	0.980	1.022
Cholesterol (mmol/l)	0.726	1.024	0.896	1.170
HDL (mmol/l)	0.246	1.411	0.789	2.525
LDL (mmol/l)	0.763	1.024	0.879	1.192
Triglycerides (mmol/l)	0.686	0.932	0.662	1.312
VLDL (mmol/l)	0.590	0.970	0.868	1.084
APOA (g/L)	0.126	1.774	0.851	3.697
APOB (g/L)	0.870	1.044	0.621	1.756
APOA/APOB	0.196	1.280	0.880	1.862
Lipoprotein A (mg/dl)	0.936	1.000	0.998	1.002
Fibrinogen (mg/dl)	0.200	1.117	0.943	1.324
Hs-CRP (mg/l)	0.894	1.001	0.987	1.016

Random blood sugar (mmol/l)	0.058	1.079	0.997	1.167
Creatinine (umol/l)	0.180	0.993	0.984	1.003
Uric acid (umol/l)	0.436	0.999	0.998	1.001
Homocysteine (umol/L)	0.001	1.012	1.006	1.018
Coronary angiogram				
Normal (%)	0.291	1.851	0.590	5.810
Triple vessel lesion (%)	0.683	0.922	0.626	1.359
Double vessel lesion (%)	0.897	1.028	0.677	1.561
Single vessel lesion (%)	0.974	1.019	0.325	3.199
Left main lesion (%)	0.186	1.617	0.793	3.299
Anterior descending branch lesion (%)	0.484	1.128	0.805	1.581
Rotatory branch lesion (%)	0.864	0.968	0.664	1.410
Lesion of right coronary artery (%)	0.501	0.888	0.627	1.256
Average number of scaffolds	0.262	1.106	0.9927	1.319
Average length of support (mm)	0.363	1.003	0.997	1.009
Medication situation				
Aspirin (%)	0.822	1.253	0.175	8.959
Clopidogrel (%)	0.540	0.775	0.342	1.755
Statins (%)	0.171	0.662	0.366	1.195
Nitrates (%)	0.820	1.039	0.746	1.448
ACEI/ARB (%)	0.450	0.882	0.638	1.221
β-blockers (%)	0.001	0.581	0.416	0.812
Calcium antagonist (%)	0.036	1.489	1.026	2.163

PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; UA, unstable angina pectoris; NSTEMI, non-ST-segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; LVEF: ejection fraction; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low density-lipoprotein; APOA, apolipoprotein A; APOB, apolipoprotein A; Hs-CRP: C-reactive protein; APOA, apolipoprotein A; APOB, apolipoprotein B; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor antagonist.

Data are presented as odds ratios (95% confidence intervals, CI).

Table 3. Multivariate Cox proportional risk model for MACE events

Multivariate COX regression				
		95%CI		
Variable	P value	HR	Bottom	Top
Age (years old)	0.325	1.024	0.977	1.074
Sex (female %)	0.006	2.546	1.303	4.975
History of hypertension (%)	0.658	1.102	0.717	1.692
History of diabetes (%)	0.010	1.827	1.153	2.895
History of cerebral infarction (%)	0.291	0.685	0.340	1.383
Smoking history (%)	0.338	1.217	0.814	1.819
Family history of CHD (%)	0.049	1.458	1.002	2.121
Past history of myocardial infarction (%)	0.743	1.128	0.548	2.320
History of PCI (%)	0.263	0.655	0.312	1.373
History of CABG (%)	0.322	2.107	0.482	9.209
Diagnosis				
UA (%)	0.118	1.513	0.900	2.542
NSTEMI (%)	0.129	1.694	0.858	3.345
STEMI (%)	0.167	1.906	0.677	2.834
LVEF (%)	0.104	0.979	0.954	1.004
Cholesterol (mmol/l)	0.931	1.038	0.445	2.423
HDL (mmol/l)	0.335	0.453	0.091	2.262
LDL (mmol/l)	0.620	0.790	0.311	2.008
Triglycerides (mmol/l)	0.534	1.744	0.056	2.732
VLDL (mmol/l)	0.555	0.914	0.678	1.232
APOA (g/L)	0.699	1.442	0.226	9.194
APOB (g/L)	0.206	4.992	0.414	60.181
APOA/APOB	0.153	2.017	0.688	4.701
Lipoprotein A (mg/dl)	0.762	1.000	0.997	1.002
Fibrinogen (mg/dl)	0.957	0.993	0.774	1.274

Hs-CRP (mg/l)	0.403	1.008	0.989	1.027
Random blood sugar (mmol/l)	0.422	0.954	0.850	1.070
Creatinine (umol/l)	0.877	0.999	0.987	1.012
Uric acid (umol/l)	0.579	1.001	0.998	1.003
Homocysteine (umol/L)	0.001	1.018	1.011	1.025
Coronary angiogram				
Normal (%)	0.300	2.941	0.382	22.643
Triple vessel lesion (%)	0.392	1.914	0.433	8.453
Double vessel lesion (%)	0.572	1.453	0.398	5.306
Single vessel lesion (%)	0.167	0.236	0.307	1.834
Left main lesion (%)	0.807	1.105	0.495	2.468
Anterior descending branch lesion (%)	0.724	1.082	0.699	1.675
Rotatory branch lesion (%)	0.749	1.089	0.645	1.839
Lesion of right coronary artery (%)	0.568	0.998	0.569	1.447
Average number of scaffolds	0.700	1.102	0.674	1.802
Average length of support (mm)	0.565	1.004	0.990	1.019
Medication situation				
Aspirin (%)	0.180	5.176	0.468	57.268
Clopidogrel (%)	0.292	0.607	0.239	1.537
Statins (%)	0.261	0.686	0.356	1.324
Nitrates (%)	0.617	0.911	0.633	1.311
ACEI/ARB (%)	0.777	0.946	0.646	1.387
β-blockers (%)	0.012	0.626	0.434	0.904
Calcium antagonist (%)	0.396	1.202	0.786	1.837

PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; UA, unstable angina pectoris; NSTEMI, non-ST-segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; LVEF: ejection fraction; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; APOA, apolipoprotein A; APOB, apolipoprotein A; Hs-CRP: C-reactive protein; APOA, apolipoprotein A; APOB, apolipoprotein B; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor antagonist.

Figures

Figure 1

MACE survival function and risk function.

Figure 2

The predictive value of Hcy for MACE risk.

Figure 3

Effect of atorvastatin on homocysteine-induced CRP expression. After pretreatment with 10 µM atorvastatin for 24 h, VSMCs were incubated in a 100 µM Hcy environment for 12 h. The expression of CRP protein (A) and mRNA (B) was detected by Western blotting and RT-PCR, respectively. Hcy (100 µM) was used as a control to which the other three groups were compared. The results are expressed as the mean ± standard deviation. * $P < 0.05$ vs. Hcy+ atorvastatin (10 µM), ** $P < 0.05$ vs. control.

Figure 4

Effect of atorvastatin on Hcy-induced ROS generation. VSMCs were treated with 10 μ M atorvastatin for 24 h, pretreated with H₂DCF-DA (10 μ M) for 30 min, and then incubated in 100 μ M Hcy for 40 min. The fluorescence intensity was measured by fluorescence microscopy. (A) Fluorescence image, (A) Control, (b) Hcy alone group, (c) Hcy+ atorvastatin (10 μ M), and (d) atorvastatin group (10 μ M). The relative fluorescence intensity was determined by ImageJ. The Hcy group alone was used as the drug control group to which the other three groups were compared, and the results are expressed as the mean $\pm$ standard deviation. ** P <0.05 vs. control, * P <0.05 vs. Hcy + atorvastatin (10 μ M).

Figure 5

Cells were pretreated with atorvastatin (10 μ M) for 24 h and then incubated in homocysteine (100 μ M) for 12 h. Western blotting was used to detect the expression of NF- κ B induced by homocysteine and assess the effects of atorvastatin. Homocysteine alone was used as the control group to which the other three groups were compared. The results are expressed as the mean $\pm$ standard deviation, ** P <0.05 vs. control, * P <0.05 vs. Hcy+ atorvastatin (10 μ M).

Figure 6

Effects of atorvastatin on IL-1 β and IL-6 expression induced by homocysteine. The cells were pretreated with atorvastatin (10 μ M) and stimulated with homocysteine (100 μ M) for 12 h. The protein and mRNA expression of IL-1 β (A, B) and IL-6 (C, D) were detected by Western blotting and RT-PCR, respectively. The homocysteine group was used as the drug control group and was compared with the control group and

the atorvastatin intervention group. The results are expressed as the mean $\pm$ standard deviation. ** $P<0.05$ vs. control, * $P<0.05$ vs. Hcy+ atorvastatin (10 μ M).