a concentration of 0.05 µg/L, we obtained long-term CVs between 12% and 20% over 12 months. As expected, long-term CVs have been higher than that obtained in our initial evaluation. During this time, we have identified 6 patients with suspected false-positive results (range, 0.1–0.6 µg/L) based on discrepancies between results and clinical information and persistently increased TnI concentrations. Normal TnI concentrations were obtained in these samples after preincubation with heterophile blocking tubes and in other troponin assays. The theoretical incidence of these heterophile antibodies in our population is 1 in 5000.

The Architect STAT Troponin-I assay provides measurement of cTnI in plasma with a CV of 10% near the 99th percentile of a reference group of healthy blood bank donors (0.03 µg/L). Our 99th percentile value is higher than that reported by the manufacturer (0.012 µg/L) and may be attributable to our use of freshly collected blood bank specimens.

Comparison with the clinically evaluated AxSYM cTnI assay (13–15) showed that the Architect STAT Troponin-I assay identified additional patients who had clinical evidence of cardiac damage. This is in keeping with studies showing that the AxSYM assay may miss patients who later developed poor cardiac outcomes (16). Furthermore, the Architect TnI showed good agreement with the Access AccuTnI at the lower end of the measurement range. Similar data comparing the Architect assay with the Advia Centaur assay were reported recently (17). Identification of such patients is important given the likely benefit from early intervention.

Although the Architect STAT Troponin-I assay was not affected by any of the substances that cause interference in the AxSYM cTnI assay, our 6 cases of heterophile antibody interference are reminders that interference from these and like substances remains an issue. Consistently increased troponin concentrations without a pattern of increase and decrease should be questioned by laboratory staff. Laboratories should have documented procedures to investigate suspicious results such as access to different troponin methodologies in real time.

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References


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Atorvastatin Reduces the Expression of COX-2 mRNA in Peripheral Blood Monocytes from Patients with Acute Myocardial Infarction and Modulates the Early Inflammatory Response, Ping Deng, Shui-ping Zhao, Hai-ying Dai, Xian-song Guan, and Hong-guang Huang (* Department of Cardiology, the Second XiangYa Hospital, Central South University, Hunan, People’s Republic of China; †Department of Cardiology, Changsha Central Hospital, Hunan, People’s Republic of China; ‡address correspondence to this author at: Department of Cardiology, Changsha Central Hospital, E-410014 Hunan, People’s Republic of China; fax 86-731-5590171, e-mail dengping2115@yahoo.com.cn)

Background: We examined the effect of atorvastatin on the expression of COX-2 in peripheral blood monocytes from patients with early stage of acute myocardial infarction (AMI), and the plasma C-reactive protein (CRP) concentrations were also examined.

Methods: Patients with AMI (n = 40) and with stable coronary heart disease (CHD; n = 18) were registered,
Inflammation plays a pivotal role in the genesis of plaque rupture, and peripheral blood monocytes (PBMs) may play a major role in the pathogenesis of atherosclerosis, which is one of the major sources of proinflammatory cytokines (1). C-reactive protein (CRP) is a nonspecific systemic inflammatory marker used for risk stratification of acute myocardial infarction (AMI) (2), which is modulated by various cytokines (3).

Prostaglandins are inflammatory mediators whose production is controlled by cyclooxygenase (COX). COX-2, an inducible isomerase, is important in inflammation, whereas COX-1 is present in most cells and is responsible for constitutive prostaglandin formation (4). COX-2 is produced by endothelial cells, smooth muscle cells, and macrophages in human atherosclerotic lesions, and it is believed to promote atherosclerosis (5). COX-2 induction is thought to occur only in cardiomyocytes in response to stress, such as ischemia, and not in healthy myocardium (6). These findings indicate that COX-2 is specifically linked to atherosclerotic inflammation, but whether activation of COX-2 is correlated with degree of atherosclerotic inflammation is unknown.

Statins act as antiinflammatory agents through an unknown mechanism, and they stabilize plaques and maintain endothelial function (7, 8). Atorvastatin may reduce recurrent ischemic events in the early phase of AMI (7), and the use of statin therapy within the first 24 h of hospitalization for AMI has been associated with a significantly lower rate of early complications and in-hospital mortality (9). In this study, we investigated whether atorvastatin has an effect on expression of COX-2 in PBMs after AMI.

We recruited 40 patients with AMI and 18 persons of similar age with stable coronary heart disease (CHD) as controls. Patients with AMI were randomized to one of the 2 groups for 1 week: group A received routine therapy including aspirin, low-molecular-weight heparins, nitroglycerin, β-blockers, angiotensin-converting enzyme inhibitors, and/or percutaneous transluminal coronary balloon angioplasty; and group B received the same routine therapy plus atorvastatin (20 mg daily). AMI was defined as a history of ischemic chest pain >30 min, characteristic electrocardiogram changes, and increased cardiac troponin I to at least twice the upper limit of the reference interval within 24 h after the onset of pain. Stable CHD was defined as clearly established CHD and no history of recent hospital admission (within the previous 3 months) for treatment of a coronary condition. CHD was defined as a history of AMI or coronary angiography showing reduction by 70% or more in area of the lumen of the coronary artery. None of the participants had chronic heart failure, insulin-dependent diabetes mellitus, or inflammatory diseases (e.g., infections, malignancies, or autoimmune diseases). None of the participants had received lipid-lowering drugs, antibiotics, salicylates, or glucocorticoids within the preceding 2 weeks.

At baseline, peripheral blood (5 mL) was drawn from all participants as described previously (10), and after 1 week of treatment, blood was again collected from patients with AMI. The study was approved by the local Institutional Review Board.

PBMs were isolated and cultured as described previously (10). Total RNA was extracted from PBMs by use of TRIzol reagent (Invitrogen Life Technologies, Gibco). RNA (1 µg) was converted into cDNA by standard techniques with a reverse transcription system (Promega). Amplification was performed as described previously (10). The reverse transcription-PCR product was visualized by 1.5% agarose gel electrophoresis. Relative intensities of bands of interest were analyzed by a Gel Doc2000 scanner (Bio-Rad) and scan analysis software, and were expressed as the absorbance ratio of COX-2 product to cyclophilin product.

Plasma concentrations of CRP were assessed with a specific sandwich ELISA manufactured by Diagnostic Systems Laboratories. All samples were analyzed in duplicate. The detection limit was 0.175 mg/L, and the intra- and interassay imprecision (as CV) was <9.0%.

All analyses were carried out with SPSS 10.0 (SPSS Software). Numerical variables are presented as the mean (SD). Because some data were skewed, they were natural log–transformed for all analyses. Comparisons between groups were analyzed by t-test (two-sided) or one-way ANOVA, followed by the Bonferroni test for experiments with more than 2 subgroups. Categorical variables were compared by use of a χ² test. The association of measurements with other biochemical variables was assessed by...
the Spearman rank correlation test. We accepted statistical significance at $P < 0.05$.

Sex distribution, age, body mass index, and blood pressure were not significantly different among the groups (Table 1). Plasma CRP concentrations were significantly higher and COX-2 expression in monocytes was increased in both groups A and B compared with the stable CHD controls, but neither plasma CRP concentration nor COX-2 expression differed significantly between groups A and B ($P > 0.05$; Table 1; Fig. 1A). The routine treatment prescription did not differ significantly between groups A and B (Table 1).

After 1 week of treatment, groups A and B both had significantly lower mean (SD) COX-2 expression [0.91 (0.13) vs 0.69 (0.14) and 0.93 (0.12) vs 0.32 (0.08), respectively] in monocytes, but the concentrations in group B decreased by 66% compared with 24% for group A ($P < 0.05$; Fig. 1). In addition, after 1 week of treatment, groups A and B both had significantly lower plasma CRP concentrations [41.8 (15.8) vs 27.3 (9.9) and 44.8 (14.0) vs 16.0 (3.2) mg/L, respectively], but in group B, concentrations decreased by 62% compared with 35% in group A ($P < 0.05$).

Expression of COX-2 mRNA in PBMs from patients with AMI was positively correlated with plasma CRP concentrations ($r = 0.662$; $P < 0.05$). Among these patients, COX-2 expression in those with AMI after 1 week of atorvastatin treatment was also positively correlated with plasma CRP concentrations ($r = 0.724$; $P < 0.05$).

These data confirm previous reports of COX-2 expression in ischemic heart disease and show for the first time that COX-2 expression is correlated with CRP concentrations in early-stage AMI. At the same time, atorvastatin might affect CRP in AMI at least partly through the COX-2–dependent pathway.

Local and systemic inflammatory responses are commonly involved in the progress of AMI. PBMs are activated after AMI, and these inflammatory cells are important in blood prone to thrombosis (vulnerable blood) (1, 8). In our study population, COX-2 expression in PBMs was increased after AMI, suggesting a potential link between COX-2 expression and AMI. We also found that COX-2 expression was closely correlated with plasma CRP concentrations in AMI, supporting a facilitative role of COX-2 in acute atherosclerotic inflammation. Chenevard et al. (11) also reported that selective COX-2 inhibition can improve endothelial function and decrease inflammation in severe coronary artery disease. COX-2 is a chemical enzyme with multiple effects. COX-2–mediated eicosanoid production by activated monocytes or macrophages might promote atherosclerosis through several

<table>
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<th>Table 1. Characteristics of participants at baseline.</th>
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<td><strong>Control group</strong></td>
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<td>Mean (SD) WBC, × 10⁹/L</td>
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<td>Mean (SD) CRP, mg/L</td>
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<td>Mean (SD) COX-2 mRNA</td>
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*NS, not significant; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, leukocytes; ACE, angiotensin-converting enzyme; LMWH, low–molecular-weight heparins; PTCA, percutaneous transluminal coronary balloon angioplasty.

b $P < 0.05$ vs controls.

c COX-2 mRNA expressed as absorbance ratio of COX-2 product to cyclophin product.

Fig. 1. COX-2 expression in PBMs from all patients. (Top), lane 0, DNA marker (pUC19DNA/Msp1/MBI); lane 1, group B posttreatment; lane 2, group B pretreatment; lane 3, group A pretreatment; lane 4, group A posttreatment; lane 5, control group. (Bottom), reverse transcription-PCR results for COX-2 mRNA are expressed as absorbance ratio of COX-2 product to cyclophin product. Posttreatment, both groups A and B had a significantly lower COX-2 expression in monocytes, but in group B it had decreased by 66%, whereas in group A it had decreased by 24% (both $P < 0.05$). Error bars, SD.
mechanisms, including activating chemotaxis, increasing vascular permeability, propagating the inflammatory cyclooxygenase cascade, and stimulating macrophage action and smooth muscle cell migration (12, 13). For example, COX-2 and its prostaglandin E₂ production have been shown to induce production of the proinflammatory cytokine interleukin-6 (13), an important cytokine that stimulates CRP production in the liver. Thus, COX-2 may promote acute inflammatory processes after AMI. Statins may decrease CRP concentrations and have effects on inflammation, plaque stabilization, and improvement of endothelial function in acute coronary syndrome. Our findings confirm that atorvastatin can decrease CRP concentrations in early-stage AMI. However, atorvastatin can inhibit COX-2 expression, which is closely correlated with CRP. This finding suggests that atorvastatin might have antiinflammatory effects at least partly through the COX-2 pathway. Hernandez-Presa et al. (14) also found that atorvastatin decreased COX-2 expression in a rabbit model of atherosclerosis and in cultured vascular smooth muscle cells. The mechanism underlying this effect of atorvastatin is probably related to inhibition of nuclear factor-κB activity secondary to a decrease in isoprenylation of proteins involved in intracellular signal transduction necessary for their correct function, because COX-2 is controlled by this transcription factor, and it has been confirmed that statins can directly decrease nuclear factor-κB activity (15).

References

Sensitive and Inexpensive Molecular Test for Falciparum Malaria: Detecting Plasmodium falciparum DNA Directly from Heat-Treated Blood by Loop-Mediated Isothermal Amplification, Leo L.M. Poon, Bonnie W.Y. Wong, Edmund H.T. Ma, Kuok H. Chan, Larry M.C. Chow, Wimal Abeysinghikreme, Nappadon Tangsupkuml, Kwok Y. Yuen, Yi Guan, Sornchai Looareesuwan, and J.S. Malik Peiris1 (1 Department of Microbiology, The University of Hong Kong, Hong Kong SAR; 2 Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong SAR; 3 Department of Parasitology and Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya, Sri Lanka; 4 Department of Clinical Tropical medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; * address correspondence to this author at: Department of Microbiology, University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong SAR; fax 852-2855-1241, e-mail lmpoon@hkucc.hku.hk)

Background: Malaria is one of the most important parasitic infections in humans. A sensitive diagnostic test for malaria that could be applied at the community level could be useful in programs to control the disease. The aim of the present work was to develop a simple, inexpensive molecular test for Plasmodium falciparum. Methods: Blood was collected from controls (n = 100) and from patients diagnosed with falciparum malaria infection (n = 102), who were recruited to the study. Heat-treated blood samples were tested by a loop-mediated isothermal amplification (LAMP) assay for P. falciparum. Results were interpreted by a turbidity meter in real time or visually at the end of the assay. To evaluate the assay, DNA from these samples was purified and tested by PCR. Results from the LAMP and PCR assays were compared.

Results: The LAMP assay detected P. falciparum directly from heat-treated blood. The quantitative data from the assay correlated to the parasite counts obtained by blood-film microscopic analyses. When we used the PCR assay as the comparison method, the sensitivity

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