Intensity of C-reactive Protein Immunohistochemical Staining of Atherosclerotic Plaque Macrophages and Extracellular Tissue of Patients with Angina Pectoris undergoing Directional Coronary Atherectomy

Tzu-Hsien Tsai, MD; Sarah Chua, MD; Cheng-Hsu Yang, MD; Chi-Ling Hang, MD; Yuan-Kai Hsieh, MD; Yen-Hsun Chen, MD; Han-Tan Chai, MD; Kuo-Ho Yeh, MD; Hon-Kan Yip, MD

Background: An elevated C-reactive protein (CRP) level plays a crucial role in cell biology of atherosclerosis and unstable plaque formation. However, direct evidence of CRP involvement in atherosclerotic plaque development and vulnerability is still limited. We hypothesized that CRP is present in the vulnerable plaques and that CRP staining intensity is stronger in vulnerable plaques compared to stable plaques.

Methods: Directional coronary atherectomy (DCA) was performed on 58 patients with stable angina (group 1) and 40 patients with unstable angina (group 2). White blood cell (WBC) counts were measured prior to DCA. Immunohistochemical staining (IHCS) was performed to localize CRP in the atheroma. Staining intensity in macrophages and extracellular tissue was graded as: 0, no staining; 1+, < 30%; 2+, 30%-60%; 3+, > 60%.

Results: The IHCS demonstrated that CRP staining ≤ 1+ intensity in macrophages and extracellular tissue were significantly higher in group 1 than in group 2 patients (all p values < 0.0001). However, IHCS demonstrated that CRP staining ≥ 2+ intensity in macrophages and extracellular tissue were significantly higher in group 2 than in group 1 patients (all p values < 0.0001). By multiple analysis, only stable angina was independently associated with CRP staining ≤ 1+ intensity in both macrophages and extracellular tissue (p < 0.0001), whereas unstable angina and WBC counts were independent predictors of CRP staining ≥ 2+ intensity in both macrophages and extracellular tissue (p < 0.0001).

Conclusion: CRP was frequently found in atherosclerotic plaques of patients with unstable angina. This analytical finding suggests that CRP directly mediates an inflammatory process in the atherosclerotic plaque.

(Chang Gung Med J 2007;30:313-20)

Key words: C-reactive protein, immunohistochemical staining, angina pectoris, obstructive coronary artery disease

From the Division of Cardiology, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan.

Received: Jun. 26, 2006; Accepted: Jan. 4, 2007

Correspondence to: Dr. Hon-Kan Yip, Division of Cardiology, Chang Gung Memorial Hospital. 123, Dapi Rd., Niaosong Township, Kaohsiung County 833, Taiwan (R.O.C.) Tel: 886-7-7317123 ext. 2363; Fax: 886-7-7322402; E-mail: tang@cgmh.org.tw

An increasing number of studies have reported that inflammation plays a crucial role in the cell biology of atherosclerosis.[1-4] Pathological and immunohistochemical staining (IHCS) studies have clearly shown abundant inflammatory cells in the ruptured plaques of patients who have died of acute coronary syndromes (ACS).[5-7] Therefore, inflammation has been suggested to play a important role in destabilizing the fibrous cap tissue, thereby enhancing risk of coronary thrombus.[1] Inflammation, manifested by elevated serum C-reactive protein (CRP) levels measured by high-sensitivity assay (hs-CRP), is associated with increased risk of cardiovascular events.[8,9] Recent studies have indicated that hs-CRP is related to adverse outcomes in ACS patients.[9,10] Even with early aggressive coronary intervention, the serum CRP level remains a strong independent predictor of short- and long-term mortality in ACS patients.[9] Taking the results of both clinical observations[9,10] and pathological findings[5-7] into consideration, a recent study[11] has suggested that hs-CRP may not only mirror inflammatory response but also directly promote atherosclerotic propagation and destabilize plaque. Therefore, elevated serum hs-CRP levels in patients with ACS may portend vulnerable plaque rupture.[7,11]

Notably, while numerous studies have documented that inflammatory cells are well appreciated in playing an important role in the immediate site of plaque rupture and erosion,[1-3,6,12] detailed information regarding the presence or absence and the role of CRP within atherosclerotic plaques is limited.[7] Thus, this study investigated CRP staining intensity by IHCS in atherosclerotic plaques to evaluate the potential role of CRP in the pathogenesis of plaque rupture. For this purpose, we examined atherosclerotic tissue obtained from patients with angina pectoris who had undergone directional coronary atherectomy (DCA).

METHODS

Patient population and exclusion criteria

Between May 1994 and April 1997, stents were still not available in Taiwan. Thus, all patients with angina pectoris and ACS were considered eligible for DCA when angiographic findings met the following criteria: (1) the reference lumen diameter of the culprit artery was ≥ 3.5 mm; (2) proximal obstruction of the left anterior descending artery (LAD), right coronary artery (RCA) or left circumflex artery (LCX); and (3) ostial lesion of LAD, RCA or LCX. Patients with a heavily calcified culprit lesion, left main coronary artery disease (CAD) or extreme tortuosity of the culprit vessel were excluded from this study. To avoid other variables that could influence the serum CRP levels in this study, the following exclusion criteria were employed: history of recent surgery or trauma in the preceeding 2 months; renal insufficiency (creatinine > 1.5 mg/dL); malignancy or liver cirrhosis; febrile disorders; acute or chronic inflammatory disease at study entry; history of recent myocardial infarction (MI) (< 21 days). Patients with a fever (body temperature > 37.5°C) prior to DCA were also excluded.

A total of 159 patients underwent DCA during the 3-year study period. Of these, 39 patients had suffered recent MI, 11 patients had a febrile disorder prior to the procedure, 7 patients had renal insufficiency and 4 patients had liver cirrhosis, were excluded. Therefore, the remaining 98 patients, including 58 patients with stable angina (group 1) and 40 patients with unstable angina (group 2), which was defined by using Braunwald's classification,[13] constituted the study population.

Blood sampling, laboratory investigations and timing of CRP staining

Blood samples were obtained prior to DCA. Measurement of white blood cell (WBC) counts, electrolytes, and biochemical analysis were done using standard laboratory methods.

Blood samples were not collected for serum hs-CRP levels as this standard method for measuring hs-CRP levels was not available in our laboratory during the study period.

Until recently, only IHCS, an analytical test developed by us, was available for CRP staining. As such, tissue samples were harvested and stored for several years prior to this investigation.

Procedure, tissue sampling, tissue processing and staining

Informed consent was obtained from all study subjects. The study protocol was approved by the Institutional Review Committee on Human Research of our institution. Right femoral arterial approach was used for all patients. AtheroCath (tm) (Guidant

Corp., Santa Clara, CA, USA) was used to rotationally cut-down the atherosclerotic plaque. The obtained atherosclerotic tissues were embedded in paraffin.

Paraffin blocks were serially sectioned at a 3 μm thickness for IHCS in each case. Two sections for each case were prepared and incubated with specific anti-CD68 antibody (Dako Corp., Carpinteria, CA, USA), diluted 1:50 for macrophage (an index of inflammatory cell) identification. Three sections were prepared in each case, and IHCS was then performed using standard avidin-biotin techniques and a commercially available antiserum for CRP (Sigma Corp., St. Louis, MO, USA) at a dilution of 1:200. Super sensitive (tm) polymer-HRP IHC was used as a detection system (BioGenex, San Ramon, CA, USA). Deparaffinized sections were incubated in 1 mmol/L EDTA buffer with steam heat before staining.

Qualitative analysis for staining

Staining intensity was graded to determine presence of CRP in macrophages and atherosclerotic tissue (extracellular staining). A qualitative score of 0-3 was applied to each sample: 0, no staining; 1+, < 30% macrophage staining or < 30% extracellular staining; 2+, 30%-60% macrophage staining or 30%-60% extracellular staining; 3+, > 60% macrophage staining or > 60% extracellular staining. Staining was performed by a pathologist who was unaware of the procedure and patient's clinical information.

Statistical analysis

Categorical variables were compared using the Chi-square test or Fischer exact test. Continuous variables were compared using the *t*-test. Logistic regression analysis was used to determine independent predictors of CRP staining intensity in macrophages and extracellular tissue. SAS statistical software for Windows version 8.2 (SAS Institute, Cary, NC, USA) was used for statistical analysis. A probability value of < 0.05 was considered statistically significant.

RESULTS

Table 1 depicts baseline characteristics, laboratory findings and angiographic results for the 98 study patients. The two groups did not differ significantly with regard to age, gender, risk factors of CAD, previous MI, prior stroke, body mass index, body temperature, creatinine level and medications. However, WBC counts were significantly higher in group 2 than in group 1 patients.

No significant differences between the two groups for multi-vessel disease, pre- and post-DCA reference lumen diameter, pre-DCA stenosis, lesion

Table 1. Baseline Characteristics, Laboratory Findings and Angiographic Results of 98 Study Patients

Variable	Group 1 (n = 58)	Group 2 (n = 40)	*p*
Age (yrs)	60.8 ± 9.7	61.2 ± 10.4	0.476
Male gender	81.0% (47)	77.5% (31)	0.760
Hypertension	46.6% (27)	50.0% (20)	0.737
Current smoking	44.8% (26)	42.5% (17)	0.820
Hypercholesterolemia	43.1% (25)	45.0% (18)	0.853
Diabetes mellitus	29.3% (17)	32.5% (13)	0.736
Previous myocardial infarction	8.6% (5)	7.5% (3)	1.0
Previous stroke	6.9% (4)	5.0% (2)	1.0
Body mass index (Kg/m^2)*	25.8 ± 4.7	25.1 ± 5.4	0.547
Body temperature (°C)	36.5 ± 0.5	36.7 ± 0.6	0.413
WBC counts (x 10^3/mL)	5.8 ± 1.2	7.9 ± 2.4	< 0.0001
Creatinine (mg/dL)	1.2 ± 0.3	1.1 ± 0.1	0.672
Aspirin	100% (58)	100% (40)	1.00
ACEIs	58.6% (34)	62.5% (25)	0.700
Statins	53.4% (31)	55.0% (22)	0.880
Multi-vessel disease†	53.5% (31)	52.5% (21)	0.926
DCA vessel			0.856
LAD	65.5% (38)	60.0% (24)	
RCA	25.9% (15)	30.0% (12)	
LCX	8.6% (5)	10.0% (4)	
Pre-PCI RLD	3.58 ± 0.47	3.61 ± 0.57	0.562
Stenosis (%)	75.6 ± 6.7	76.7 ± 5.9	0.231
Lesion length (mm)	12.3 ± 5.8	11.2 ± 4.7	0.113
Post-PCI MLD	3.19 ± 3.3	3.26 ± 4.2	0.345
Post-PCI RLD	3.67 ± 0.37	3.71 ± 0.44	0.221

Abbreviations: ACEIs: angiotensin converting enzyme inhibitors; DCA: directional coronary atherectomy; LAD: left anterior descending artery; LCX: left circumflex artery; MLD: minimal lumen diameter; PCI: percutaneous coronary intervention; RCA: right coronary artery; RLD: reference lumen diameter; WBC: white blood cell; Data are expressed as mean value ± SD or % (no.) of patients; *: Body mass index was defined as the weight in kilograms divided by the square of the height in meters (kg/m^2); †: Multi-vessel disease was defined by stenoses of > 50% in ≥ 2 major epicardial coronary arteries.

length, and pre- and post-DCA minimal lumen diameter were demonstrated by angiographic findings. Additionally, DCA performed on the LAD, RCA or LCX in both groups was similar.

Immunohistochemical staining (Table 2, Fig. 1 and Fig. 2)

Table 2 shows the IHCS results for both groups. The IHCS demonstrated that CRP staining ≤ 1+ intensity in macrophages and extracellular tissue were significantly higher in group 1 than in group 2 patients (all *p* values < 0.0001). However, CRP staining ≥ 2+ intensity in macrophages was significantly higher in group 2 than in group 1 patients. Furthermore, CRP staining ≥ 2+ intensity in extracellular tissue was markedly stronger in group 2 compared to that in group 1.

Multiple stepwise logistic regression analysis (Table 3) of baseline variables (Table 1), and clinical presentations of unstable angina and stable angina demonstrated that stable angina is the only independent predictor of CRP staining ≤ 1+ intensity in both macrophages and extracellular tissue. Conversely, unstable angina was independently associated with CRP staining ≥ 2+ intensity in both macrophages and

Table 2. Immunohistochemical C-reactive Protein Staining Intensity of Macrophages and Extracellular Tissue

Variables	Group 1 (n = 58)	Group 2 (n = 40)	*p*
Grading of CRP staining in macrophages			< 0.0001
Grade 3+	3.4% (2)	25.0% (10)	
Grade 2+	5.2% (3)	40.0% (16)	
Grade ≤ 1	91.4% (53)	35.0% (14)	
Grading of CRP staining in extracellular tissue			< 0.0001
Grade 3+	6.9% (4)	50.0% (20)	
Grade 2+	13.8% (8)	40.0% (16)	
Grade ≤ 1	79.3% (46)	10.0% (4)	

Abbreviation: CRP: C-reactive protein.
Data are expressed as % (no.) of patients.

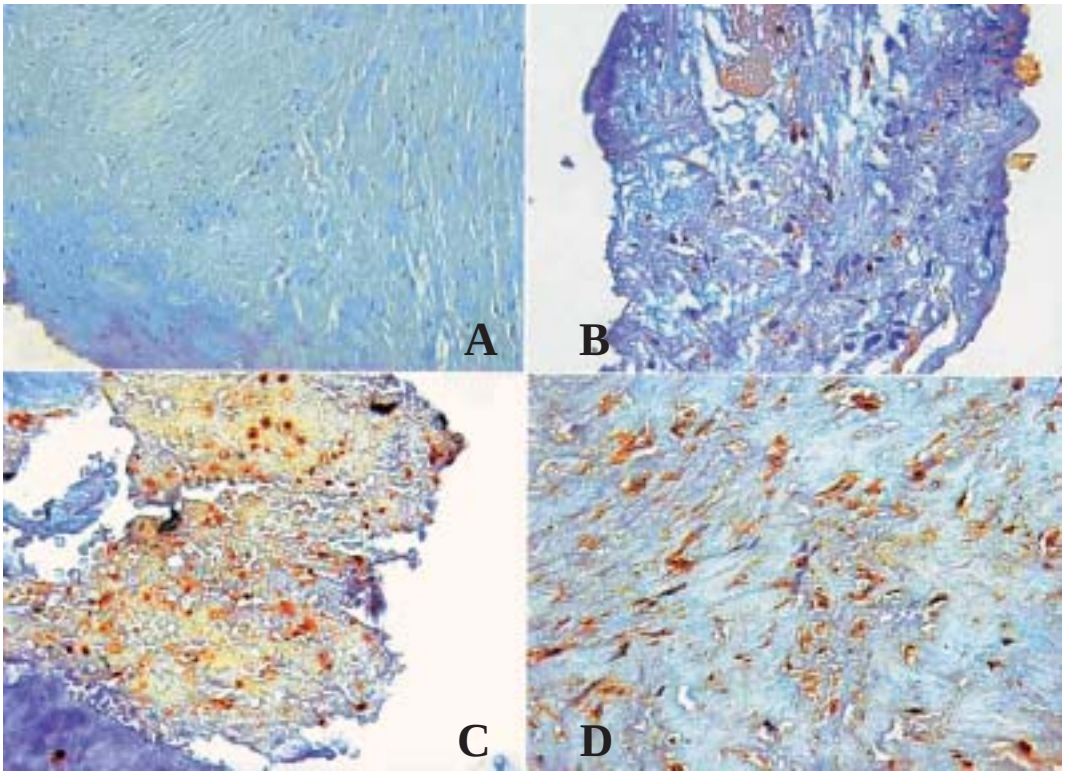

Fig. 1 Immunolocalization and C-reactive protein staining intensity in atherosclerotic tissue section (original magnification x 132). Dark brown color staining indicates grade zero (A), grade 1+ (B), grade 2+ (C) and grade 3+ (D) CRP staining intensity within both macrophages and extracellular tissue.

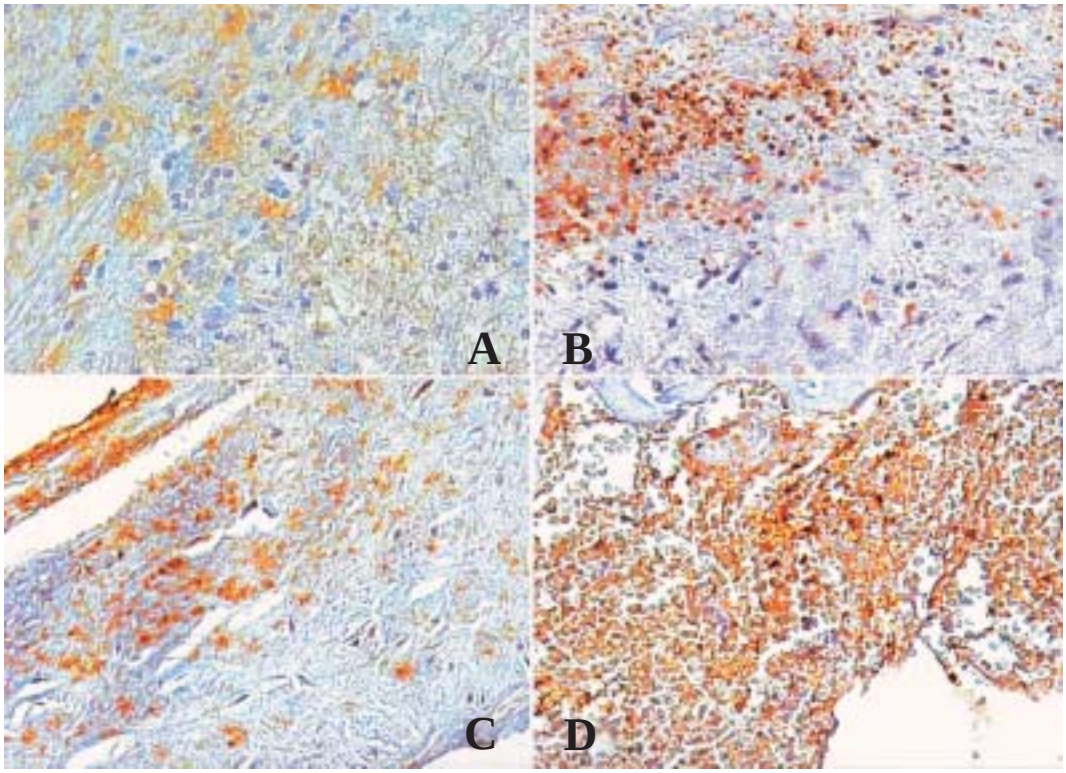

Fig. 2 Immunolocalization and C-reactive protein (CRP) staining intensity in atherosclerotic tissue section (original magnification x 132; dark brown color indicates positive CRP staining). (A) Indicates grade 3+ CRP staining within extracellular tissue and grade zero CRP staining within macrophages. (B) Indicates grade 3+ CRP staining within macrophages and grade zero CRP staining within extracellular tissue. (C) Indicates grade 3+ CRP staining within extracellular tissue and grade 1+ CRP staining within macrophages. (D) Indicates grade 3+ CRP staining within macrophages and grade 1+ CRP staining within extracellular tissue.

Table 3. Independent Predictors of ≤ 1 or ≥ 2 C-reactive Protein Staining Intensity of both Macrophages and Extracellular Tissue

Variables	OR	95% CI	*p*
For ≤ 1 grading of CRP staining			
Stable angina	5.48	4.47–16.3	< 0.0001
For ≥ 2 grading of CRP staining			
Unstable angina	4.87	4.7–12.3	< 0.0001
White blood cell count	3.3	4.65–21.12	< 0.0001

Abbreviations: CI: confidence interval; CRP: C-reactive protein; OR: odds ratio

extracellular tissue. WBC count was also independently associated with CRP staining ≥ 2+ intensity in both macrophages and extracellular tissue.

DISCUSSION

To the best of our knowledge, this study was one of the largest cohort studies with a large sample size to investigate CRP staining intensity in atherosclerotic tissues of patients with angina pectoris undergoing DCA. This study provided several striking clinical implications. First, stable angina was independently correlated with absence of CRP and cellular staining in atherosclerotic tissue. Second, unstable angina was independently associated with increased CRP staining intensity in macrophages and extracellular tissue. Finally, circulating WBC count was strongly related to increased CRP staining intensity in macrophages and extracellular tissue.

Atherosclerosis, the principle cause of heart attack, stroke and peripheral obstructive artery diseases, accounts for the majority of all mortality in developed countries.[14] Atherosclerosis, formerly considered a bland lipid storage disease, actually involves an ongoing inflammatory response.[12] Growing evidence has demonstrated that inflamma-

tion has a fundamental role in mediating all stages of atherosclerosis from initiation through progression and ultimately the thrombotic complication of atherosclerosis.[1-3,5,6,12,15] Previous studies have shown that in the genesis of atherosclerotic lesions, the endothelium can interact with macrophages, platelets, smooth muscle and T lymphocytes.[14] Recent advances in basic science have further demonstrated that CRP, an index of inflammation, directly participates in the proatherosclerotic effect.[4,16] However, little is known about CRP colonization in atherosclerotic plaques of patients with obstructive CAD. In this study, one notable finding was that CRP could be identified by IHCS in macrophages and atherosclerotic tissue. Thus, analytical results of this ex vivo study, which provide useful information on CRP distribution in obstructive CAD, further support experimental results of previous in vitro studies indicating that CRP has a direct role in the pathogenesis of atherosclerosis.[4,16]

The most important finding in this study was that unstable angina is significantly and independently associated with increased CRP staining intensity in macrophages and extracellular tissue. Additionally, this study found that circulating WBC counts, an index of an inflammatory marker, were significantly higher in unstable angina patients than in stable angina patients. Furthermore, the circulating WBC count was independently related to increased CRP staining intensity in macrophages and extracellular tissue. Accordingly, we suggest that CRP and inflammatory cells, directly and continuously, mediate an inflammatory process on the fibrous cap and within the atherosclerotic plaque. Such inflammation subsequently causes plaque to rupture in the obstructed coronary artery, which was followed by a clinical setting of unstable angina in these study. A recent autopsy study by Burke et al. identified a strong correlation between hs-CRP levels and increased numbers of thin atheromas in the coronary tree.[7] Moreover, Burke et al. identified a positive correlation between the CRP staining intensity in plaque and hs-CRP serum levels.[7] They, therefore, suggested that hs-CRP levels were highest in plaques with acute rupture and erosion. Our findings collaborate with those obtained by Burke et al.[7] and, therefore, further support a recent clinical observational study[11] suggesting that hs-CRP may not only mirror an inflammatory stimulus but also directly promote atherosclerotic propagation and destabilization of plaque. Our findings may also explain why previous pathological and immunohistochemical staining studies of samples from patients who died of acute MI demonstrated that the immediate site of plaque rupture or erosion was always marked by an inflammatory process.[1,6,7]

Another primary finding in this study was that the clinical setting of stable angina was the only independent predictor of the absence of CRP and macrophage staining within extracellular tissue. This finding may reflect the fact that only mild-event absent-inflammatory reaction exists within atherosclerotic plaque. Previous studies have shown that stable plaque typically has a relatively thick fibrous cap and minimal lipid content in the core.[17] Conversely, vulnerable plaque typically has a thin fibrous cap, large amount of lipid content in the core[17,18] and an ulceration or fissuring erosion over the neck of the fibrous cap.[1,6,7,18] Our findings, based on clinical observations of patients with unstable angina, stable angina and IHCS, further confirm the previous histological and molecular-based studies.[1,6,7,17,18]

A positive correlation between serum hs-CRP level and untoward clinical outcome in patients with ACS undergoing percutaneous coronary intervention has been reported.[9,11] Growing evidence suggests that statins have an anti-inflammatory property other than lowering cholesterol levels.[9,19] Additionally, high-dose statin can strongly decrease the circulating CRP levels.[20] Accordingly, our findings, based on IHCS study, provide useful clinical information and encourage the use of statins for patients with obstructive CAD.

This study has several limitations. First, without measuring serum hs-CRP levels, no data was provided regarding the relationship between serum hs-CRP level and CRP staining intensity within the atherosclerotic plaque. Second, the high degree of sensitivity of intravascular ultrasound may reinforce the claim of an association between morphologies of atherosclerotic plaques and the clinical settings of unstable and stable angina. Hence, the sensitivity of the atherosclerotic plaque stability diagnosis (e.g. stable or vulnerable plaque) may have been less than optimal.

In conclusion, CRP was frequently identified by IHCS in the inflammatory cells and atherosclerotic

tissue of patients with unstable angina undergoing DCA. These experimental findings suggest that CRP directly participates in the inflammatory response in atherosclerotic plaques.

Acknowledgements

This study was supported by a program grant from Chang Gung Memorial Hospital, Chang Gung University (grant no. CMRPG 83004). All authors have no commercial associations, such as consultancies, stock ownership, other equity interests or patent-licensing arrangements. There is no conflict of interest in this study.

REFERENCES

1. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 1994;89:36-44.
2. Libby P, Geng YJ, Sukhova GK, Simon DI, Lee RT. Molecular determinants of atherosclerotic plaque vulnerability. Ann N Y Acad Sci 1997;811:134-45.
3. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999;340:115-26.
4. Pasceri V, Chang J, Willerson JT, Yeh ET. Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cell by anti-atherosclerosis drugs. Circulation 2001;103:2531-4.
5. Falk E, Shah PK, Fuster V. Coronary plaque disruption. Circulation 1995;92:657-61.
6. Burke AP, Farb A, Malcom GT, Liang YH, Smialek J, Virmani R. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. N Engl J Med 1997;336:1276-82.
7. Burke AP, Tracy RP, Kolodgie F, Malcom GT, Zieske A, Kutys R, Pestaner J, Smialek J, Virmani R. Elevated C-reactive protein values and atherosclerosis in sudden coronary death: association with different pathologies. Circulation 2002;105:2019-23.
8. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, Hutchinson WL, Pepys MB. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 1999;99:237-42.
9. Mueller C, Buettner HJ, Hodgson JM, Marsch S, Perruchoud AP, Roskamm H, Neumann FJ. Inflammation and long-term mortality after non-ST elevation acute coronary syndrome treated with a very early invasive strategy in 1042 consecutive patients. Circulation 2002;105:1412-5.
10. Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. N Engl J Med 2000;343:1139-47.
11. Yip HK, Wu CJ, Chang HW, Yang CH, Yeh KH, Chua S, Fu M. Levels and values of serum high-sensitivity C-reactive protein within 6 hours after the onset of acute myocardial infarction. Chest 2004;126:1417-22.
12. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135-43.
13. Braunwald E. Unstable angina. A classification. Circulation 1989;80:410-4.
14. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801-9.
15. Hansson GK. Immune and inflammatory mechanisms in the development of atherosclerosis. Br Heart J 1993;69:S38-41.
16. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation 2000;102;2165-8.
17. Libby P. Molecular bases of the acute coronary syndromes. Circulation 1995;91:2844-50.
18. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 1995;92:1355-74.
19. Nissen SE, Tuzcu EM, Schoenhagen P, Crowe T, Sasiela WJ, Tsai J, Orazem J, Magorien RD, O'Shaughnessy C, Ganz P; Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) Investigators. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. N Engl J Med 2005;352:29-38.
20. van de Ree MA, Huisman MV, Princen HMG, Meinders AE, Kluft C; DALI-Study Group. Strong decrease of high sensitivity C-reactive protein with high-dose atorvastatin in patients with type 2 diabetes mellitus. Atherosclerosis 2003;166:129-35.

比較穩定型及不穩定型心絞痛病患其粥狀斑塊中吞噬細胞內與細胞外的 C-反應蛋白免疫組織化學染色強度

蔡子賢 蔡慧玲 楊正旭 洪志凌 謝元凱 陳彥勳 柴漢東 葉國和 葉漢根

背 景： C-反應蛋白 (C-reactive protein) 在粥狀動脈硬化 (atherosclerosis) 及形成不穩定斑塊 (unstable plaque) 的細胞生物反應扮演重要的角色。然而，罕有證據顯示發炎蛋白直接參與粥狀動脈硬化的形成及斑塊的不穩定化。本研究假設發炎蛋白在不穩定斑塊內存在且其強度比在穩定斑塊 (stable plaque) 內高。

方 法： 58 位穩定型心絞痛 (stable angina) 及 40 位不穩定型心絞痛 (unstable ngina) 的病人在接受定向冠狀動脈粥狀切除術 (Directional coronary atherectomy) 治療前，接受抽血測量其血中的白血球及普通生化值。然後接受定向冠狀動脈粥狀切除術，所獲的粥狀硬化斑進行 C-反應蛋白的免疫組織化學染色 (Immunohistochemical staining) 檢查。在吞噬細胞 (macrophage) 內及細胞外組織免疫組織化學染色強度分爲四級：grade 0, 無染色強度；1+，30%；2+， 30%-60%；and 3+，> 60%。

結 果： 穩定型心絞痛病人其粥狀斑塊中吞噬細胞內外的 C-反應蛋白免疫組織學染色強度顯示 ≤ grade 1 的比例高過不穩定型心絞痛的病人 (p 值 < 0.0001)。但是，不穩定型心絞痛的病人其粥狀斑塊中吞噬細胞內外的發炎蛋白免疫組織化學染色強度顯示 ≥ grade 2 的比例高過穩定型心絞痛的病人 (p 值 < 0.0001)。由多變數分析發現穩定型心絞痛是 C-反應蛋白免疫組織化學染色強度 ≤ grade 1 的獨立預測值 (p 值 < 0.0001)，而不穩定型心絞痛和血液中白血球數目是 C-反應蛋白免疫組織化學染色的強度 ≥ grade 2 的獨立預測值 (p 值 < 0.0001)。

結 論： 不穩定型心絞痛病人其粥狀硬化斑塊經常發現 C-反應蛋白的存在，由分析的結果可以猜測 C-反應蛋白可能促使斑塊的不穩定化而後破裂產生。

(長庚醫誌 2007;30:313-20)

關鍵字：C-反應蛋白，免疫組織化學染色，心絞痛，冠狀動脈阻塞

長庚紀念醫院 高雄院區 心臟內科；長庚大學 醫學院
受文日期：民國95年6月26日；接受刊載：民國96年1月4日
通訊作者：葉漢根醫師，長庚紀念醫院 心臟內科。高雄縣833鳥松鄉大埤路123號。Tel: (07)7317123轉2363; Fax: (07)7322402; E-mail: tang@cgmh.org.tw