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Effects of C-reactive Protein and Cholesterol on Responsiveness In Vitro of the Thoracic Artery to Angiotensin II in Patients having Coronary Artery Bypass Gafting

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Abstract

The renin-angiotensin system is critically involved in the pathogenesis of atherosclerosis. Cholesterol increases the vascular responsiveness to angiotensin II by upregulation of the angiotensin II type 1 receptor. However, the effects of C-reactive protein (CRP) on vascular responsiveness to angiotensin II are unknown. We evaluated the in vitro vascular responsiveness to angiotensin II of the internal thoracic artery from patients undergoing coronary bypass grafting. We observed that both increased pre-operative CRP and increased cholesterol levels were strongly related to increased vascular responsiveness to angiotensin II ($p<0.001$ for both). However, angiotensin II mediated contraction was only increased pronounced when both the levels of CRP and cholesterol were jointly increased. In conclusion, our results suggest that CRP and cholesterol act synergistically on vascular responsiveness to angiotensin II.

Introduction

The renin-angiotensin system is critically involved in the pathogenesis of atherosclerosis. Most of its detrimental effects are mediated through activation the angiotensin type 1 receptor.[1] In vitro, exposure of vascular smooth muscle cells to cholesterol can markedly augment angiotensin type 1 receptor mRNA and protein expression.[2;3] In a randomized clinical trial, we recently demonstrated that the vascular responsiveness to angiotensin II is reduced after statin treatment.[4] We also demonstrated that the angiotensin II mediated responses were strongly related to serum cholesterol levels. In addition, in vitro exposure of C-reactive protein (CRP) also can lead to upregulation of the angiotensin type 1 receptor, and is related to increased neointimal formation in the rat carotid artery angioplasty model.[5] However, whether the effects of CRP modify human vascular responsiveness to angiotensin II remains to be determined. Therefore, we studied the effects of CRP on vascular responsiveness to angiotensin II in thoracic arteries of a cohort of patients having coronary artery bypass grafting (CABG). In addition, we studied the possible additive effects of CRP with cholesterol on these vascular responses.

Methods

Segments of the human internal thoracic arteries were collected during elective CABG for stable angina in 24 consecutive patients in the University Medical Center Groningen of the University of Groningen, the Netherlands. Patient characteristics are presented in Table 1. Segments of the internal thoracic artery were immediately washed and transported to the laboratory in ice-cold Krebs solution. Vascular responsiveness to angiotensin II was assessed using isotonic displacement transducers studies, as described previously and expressed as percentage of the maximal constriction to phenylephrine afterwards.[6] High sensitive CRP was determined by nephelometry with a threshold of 0.175 mg/L and intra- and inter-assay coefficients of < 4.4 and 5.7%, respectively (BNIIN, Dade Behring, Marburg, Germany). Data are presented as mean±SD or as indicated. Differences between group were tested with the Student t test and comparisons between complete concentration-response curves were made by repeated measure analysis of variance. The non-normally distributed CRP was transformed by natural logarithm to assess Pearson correlation coefficients (verified nonparametrically by Spearman's correlation) with cholesterol. A two-tailed P value of $\leq$0.05 was interpreted as indicating a statistical significant difference. All analyses were performed using SPSS version 12.0 software (SPSS, Chicago, IL, USA).

Results

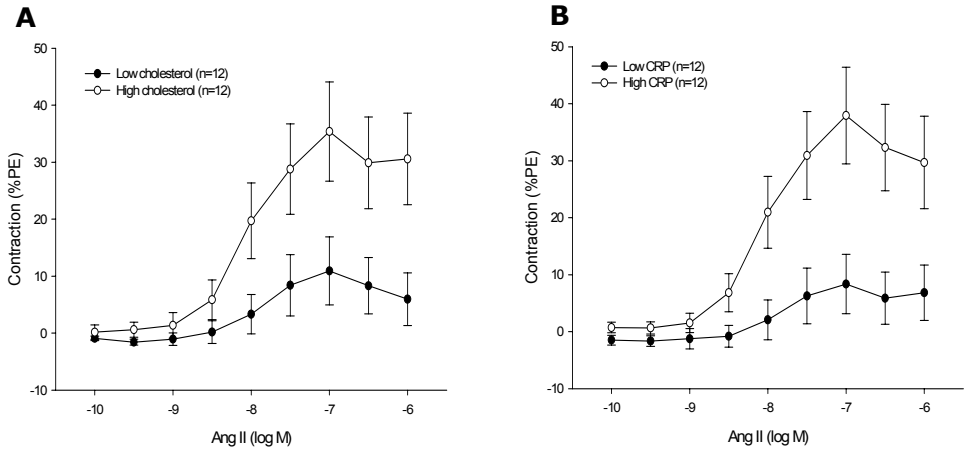
Patients characteristics are summarized in table 1. As a cutoff level for CRP and cholesterol we used the median value, 1.27 mg/l for CRP and 4.55 mmol/l (176 mg/dl) for total cholesterol. Figure 1 illustrates the significant increased vascular responsiveness to angiotensin II in the high total cholesterol group ($p<0.0001$ for repeated measurement). The maximal response to angiotensin II was also more pronounced in the higher total cholesterol group compared to the lower (40±27 versus 13±20 % phenylephrine, respectively; $P<0.05$). CRP levels above the median were also associated with an increased vascular responsiveness to angiotensin II as is illustrated in figure 1 ($p<0.0001$). When the maximal response to angiotensin II was considered, this was also augmented in patients with higher CRP levels (40±29 versus 13±17 % phenylephrine, respectively; $P<0.05$).

Table 1. Patient characteristics

Variable	N=24
Age, (years)	61 ± 10
Men/women	21/3
Hypertension	10 (42%)
Smoker	
Past	15 (63%)
Current	5 (21%)
Diabetes Mellitus	4 (17%)
Blood pressure (mm Hg)	
Systolic	131 ± 17
Diastolic	77 ± 10
Body Mass Index (kg/m^2)	27 ± 4
Canadian Cardiovascular Society (CCS) classification for angina severity	
Class I	7 (29%)
Class II	13 (54%)
Class III	4 (17%)
History of myocardial infarction	5 (21%)
Medication	
β Blockers	21 (88%)
Ca-channel antagonist	16 (67%)
Nitrates	19 (79%)
Statins	20 (83%)
Lipids, mmol/l (mg/dl)	
Total Cholesterol	4.65 ± 1.41 (180 ± 54)
Low Density Lipoprotein Cholesterol	2.86 ± 1.14 (110 ± 44)
High Density Lipoprotein Cholesterol	1.01 ± 0.24 (39 ± 9)
Triglycerides	1.73 ± 0.81 (153 ± 72)
C-reactive protein (mg/l)	1.27 [0.47-5.38]

Data are means±standard deviations for continuous variables. For C-reactive protein, the median and interquartile range are shown.

Figure 1



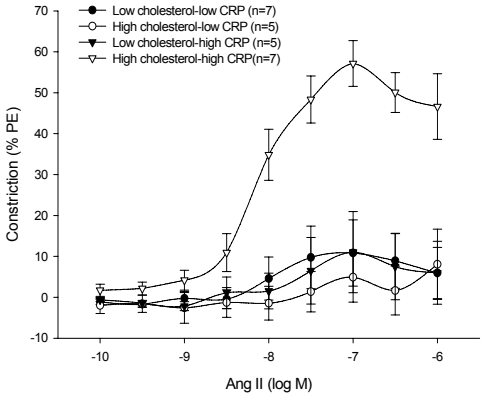
Cumulative angiotensin II dose-response curve of human thoracic artery between patients above and below the median of A.) cholesterol (median value 4.55 mmol/l or 164 mg/dl) B.) C-reactive protein (median value 1.26 mg/l).

There was not a significant correlation between cholesterol and CRP levels ($r=0.123$; $p=0.473$). Interestingly, analyzing additive effects of total cholesterol and CRP vascular responsiveness to angiotensin II was most pronounced in patients with both total cholesterol and CRP above the median value (figure 2; $p<0.0001$) indicating an important effect modification (interaction). Indeed, this finding was also significant ($p<0.01$) when both tested as a continuous variable. When testing low density lipoprotein cholesterol, instead of total cholesterol we obtained very similar results. However, high density lipoprotein was not related to vascular responsiveness to Angiotensin II.

Discussion

The current study suggests that increased CRP and increased cholesterol levels are interacting to increase human vascular responsiveness to angiotensin II.
Elevated cholesterol levels constitute a major cardiovascular risk factor in men and women at all ages.[7] Accumulating data indicates a link between lipids and the renin-angiotensin system.[2-4;8] In cell cultures, cholesterol can upregulate the angiotensin type 1 receptor and consequently increase the response to angiotensin II.[3] In hypercholesterolemic subjects, angiotensin type 1 receptor expression of platelets is increased compared to normocholesterolemic subjects.[8] Moreover, angiotensin type 1 receptor expression and vascular responsiveness can be normalized by lipid lowering therapy.[4;8] Inflammation is another hallmark in the development and progression of atherosclerosis.[9] Increased CRP levels are related to long-term prognosis in patients with documented coronary artery disease[10] and in apparently healthy men.[11] More recently, CRP is emerging as a proatherogenic mediator.[12] Currently, known proatherogenic effects of CRP are activation of vascular smooth muscle cells[13],

Figure 2.



Cumulative angiotensin II dose-response curve of the human thoracic artery among patients with C-reactive protein (CRP) and cholesterol levels above or below the median for the study population.

upregulating of the expression of adhesion molecules on endothelial cells[14;15], and mediating cholesterol uptake by macrophages[16]. In parallel with cholesterol, CRP upregulates the angiotensin type 1 receptor, at least in cell cultures and rabbits.[5] Interestingly, the mechanism by which cholesterol and CRP upregulates the angiotensin type 1 receptor differs. Cholesterol upregulates the angiotensin type 1 receptor through mechanisms that involve post-transcriptional mRNA stabilization.[3] On the other hand, CRP causes increase in angiotensin type 1 receptor mRNA expression, with no change in angiotensin type 1 receptor stability.[5] It is conceivable that these different molecular mechanisms act synergistically on angiotensin type 1 receptor expression when both cholesterol and CRP levels are increased. Importantly, cholesterol as well as CRP increases angiotensin type 1 receptor number without changing receptor affinity for angiotensin II.[3;5]

We now provide evidence that the joint effects of cholesterol and CRP on angiotensin type 1 receptor expression profoundly increase the vascular responsiveness to angiotensin II in humans. The present study is in line with a recent report evaluating CRP and cholesterol in the prediction of first cardiovascular events.[17] The combined assessment of both CRP and cholesterol proved to be superior to predict future cardiovascular events than either biological marker alone. Event free survival was worse in the above median CRP – above median low density lipoprotein cholesterol compared to the other 3 groups with one, or both, variables below median. It is tempting to speculate that part of the predictive value of combined evaluation of both CRP and cholesterol can be explained by their joint effects on the renin-angiotensin system.

A limitation of the present study was its sample size, making it susceptible for biases (e.g. use of co-medication). Furthermore, serum cholesterol and CRP levels were only measured once, the day before surgery. We only demonstrated an association between CRP, cholesterol and vascular responsiveness to angiotensin II.

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