Blood Pressure, LDL Cholesterol, and Intima-Media Thickness

A Test of the “Response to Injury” Hypothesis of Atherosclerosis

Ping Sun, Kathleen M. Dwyer, C. Noel Bairey Merz, Wei Sun, C. Anderson Johnson, Anne M. Shircore, James H. Dwyer

Abstract—The “response to injury” hypothesis is a plausible model of the development of atherosclerosis supported by observations from animal models. The present study uses epidemiological data to investigate the hypothesis that wall damage due to hypertension is a precursor of low density lipoprotein cholesterol (LDL-C)–mediated atherosclerosis. The Los Angeles Atherosclerosis Study is following a cohort of 576 participants who were aged 40 to 60 years and were free of symptomatic cardiovascular disease at recruitment. Common carotid artery intima-media thickness (IMT) was assessed by B-mode ultrasonography. After exclusion for nonfasting blood draw and other missing data, 511 subjects were available for analysis. IMT was regressed on LDL-C within tertiles of systolic blood pressure (SBP): low (93 to 122 mm Hg), middle (123 to 132 mm Hg), and high (133 to 175 mm Hg). Covariates were age, sex, body height, body mass index, ethnicity, smoking status, diabetes, and pharmacological treatment for hypertension or hypercholesterolemia. IMT was significantly related to LDL-C in the high SBP group ($\beta=0.025\pm0.008$, where $\beta$ values are IMT [mm]/LDL-C [mmol/L]; $P=0.002$) but not in the middle ($\beta=-0.006\pm0.008$, $P=0.39$) or low ($\beta=-0.004\pm0.009$, $P=0.64$) SBP group. The slope in the high SBP group was significantly greater than in the middle ($P=0.004$) or low ($P=0.014$) SBP group. Results were similar for women and men, and after the exclusion of diabetics and persons using antihypertensive or lipid-lowering medications. Elevated LDL-C was associated with increased IMT in the upper tertile of SBP but not in the lower tertiles. These findings are consistent with the hypothesis that wall injury due to elevated SBP increases the susceptibility of the artery wall to LDL-C–mediated atherogenesis. (Arterioscler Thromb Vasc Biol. 2000;20:2005-2010.)

Key Words: atherosclerosis ■ response-to-injury model ■ intima-media thickness ■ LDL cholesterol ■ blood pressure

According to the “response-to-injury” model of atherogenesis,1,2 various factors, which include hemodynamic forces and chemical agents, induce dysfunctional alterations in the overlying endothelium. This injury may then be followed by the aggregation of platelets, oxidized lipids, and smooth muscle cells in the intimal layer and by the eventual formation of plaques.

This model of atherogenesis predicts that the atherosclerotic deposition of LDL cholesterol (LDL-C) may require previous damage to the endothelium by a factor such as hypertension. There are various experimental results from animal models of atherosclerosis that support the injury hypothesis.3 For example, in the Watanabe heritable hyperlipidemic rabbit, plasma lipoproteins play a key role in determining the intimal response to hypertension.4 Hypertension-induced changes in the intima lead to thickening but do not generally progress to atherosclerotic plaque formation in the absence of elevated plasma lipoproteins.5

The roles of high blood pressure and LDL-C proposed in the response-to-injury hypothesis of atherosclerosis can be tested with B-mode ultrasound measurement of intima-media thickness (IMT) in the common carotid artery.6 Increased IMT is characteristic of natural aging7 and early atherosclerosis.2 Such thickening of the common carotid arteries has been related prospectively to the risk of coronary heart disease events.8–10 In addition, carotid IMT has been related to cardiovascular risk factors in epidemiological studies,11,12 and it has shown regression in lipid-lowering intervention trials.13–17

Epidemiological data from cohorts with coronary disease morbidity or mortality events as end points may not detect an interaction between blood pressure and blood lipid levels because of the additional role of hypertension or hypercholesterolemia in the thromboembolic pathways that lead to events.

Hypertension and elevated serum LDL-C are established as independent risk factors for thicker carotid artery intima-
media layers.\textsuperscript{8,11,12} However, we know of no published epidemiological data assessing the interaction between blood pressure and LDL-C as they relate to carotid artery wall thickness. The present study evaluates this hypothesis by investigating interactive relations between systolic blood pressure (SBP), LDL-C, and carotid artery IMT in a cross-sectional epidemiological study.

Methods

Subjects

The Los Angeles Atherosclerosis Study is a longitudinal investigation of risk factors for atherosclerosis in 576 utility company employees aged 40 to 60 years (44 to 60 years among women) at the time of recruitment who reported no history of cardiovascular disease (myocardial, angina pectoris, stroke, or revascularization). Data for the present analysis were taken from the baseline examination during 1995 to 1996. Age at examination ranged from 41 to 62 years in men and 44 to 61 years in women. Participants were randomly sampled from employees, with oversampling of Hispanics and smokers and a participation rate of 84%. All participants signed an informed consent approved by the Institutional Review Board of the Keck School of School of Medicine of the University of Southern California. Three subjects were excluded from the present analysis because of missing IMT; 53 subjects were excluded from the present analysis because of nonfasting blood draw (<8 hours) or serum triglycerides >3.955 mmol/L; and 2 subjects were excluded from the present analysis because of missing lipid measurements. Repeated measurement of lipids resulted in the exclusion of 5 subjects because of large discrepancies (difference >130 mg/dL) in total cholesterol; 2 subjects were excluded for large discrepancies (difference >40 mg/dL) in HDL cholesterol. Analyses were performed in the resulting sample of 511 and in a secondary sample of 413, which excluded 98 additional subjects who reported a history of diabetes or current use of prescribed medication for the treatment of hypertension or hypercholesterolemia.

Measures

Measures at the baseline examination included the following: ultrasound scanning of the left and right carotid arteries in 2 body positions (supine and lateral); a questionnaire concerning demographic information, medication use, and health behaviors; venipuncture; blood pressure in the brachial artery of the right arm; body size; and three 24-hour recalls of dietary intake. All measures (except 2 of the 24-hour dietary recalls) were collected in a single examination conducted in a specially equipped van that was driven to work sites. Carotid B-mode images were obtained with a portable ultrasound scanner (ATL Ultramark 4+) equipped with a 7.5-MHz linear array transducer. IMT was calculated offline with a computerized pattern recognition algorithm.\textsuperscript{18} These procedures and the reproducibility of the measurements have been reported elsewhere.\textsuperscript{19} Briefly, IMT is averaged over a 1-cm segment of the far wall of the common carotid artery 0.25 cm proximal to the carotid bulb. The number of pixels can range from 55 to 80 over the 1-cm segment, depending on the penetration depth (~55 pixels for 60-mm scanning depth, 80 for 40-mm depth) of the far wall of the artery. IMT is determined by measures on 2 frames in each of 2 body positions (lateral and supine) in the left and right arteries. The overall IMT measure was expected to be the mean of 8 frames. Out of the total of 576 subjects, IMT could not be measured on all 8 frames for 3 subjects, IMT was measurable in 4 frames for 3 subjects, and IMT was measurable in 6 frames for another 20 subjects. In the case of missing frames, IMT was calculated as the average of the available measures. By use of this protocol, the standard deviation of differences between repeated measures of IMT by different sonographers was 0.029 mm.\textsuperscript{19}

Seated and supine blood pressures were measured twice in the brachial artery with a standard mercury sphygmomanometer. The first reading occurred before the ultrasound examination; the second occurred after the examination. The 2 seated readings were averaged for analysis. Seated blood pressures were not measured for 10 of the 576 subjects in the study; these 10 missing seated blood pressure values were imputed from the supine readings.

LDL-C levels were determined from fasting serum samples. Fasting was defined as a self-reported interval of >8 hours since the last intake of food. Blood was processed immediately and stored at -20°C for 1 to 5 days; samples were then stored at -70°C until analysis. Serum lipids were determined by automated clinical chemistry analyzers. Serum LDL-C was estimated from total cholesterol, HDL cholesterol, and triglycerides by using the formula of Friedewald et al.\textsuperscript{20} LDL-C was not determined in subjects with fasting serum triglyceride levels >3.955 mmol/L.

Statistical Analysis

To depict the general relations between IMT and serum LDL-C and SBP, covariate-adjusted mean IMT was estimated within subgroups of LDL-C and SBP. Subjects were categorized into tertiles of SBP and then quintiles of LDL-C within each blood pressure group. Means within these 15 groups were adjusted for age, sex, ethnic group (non-Hispanic white, Hispanic, black, Asian, and other), body height, body mass index, smoking status (current, former, and never), diabetes (non-insulin-dependent diabetes mellitus or insulin-dependent diabetes mellitus/other), use of pharmaceutical agents for hypertension (yes/no), and hypercholesterolemia (yes/no). In the figure, mean IMT is plotted against the median of LDL-C (LDLM) within each of the 15 groups.

To estimate the linear relationship between IMT and LDL-C within SBP tertile groups and the difference of the linear dependence of IMT on LDL-C between SBP levels, a linear regression was performed in which the dependent variable was IMT. The independent variables were LDLM, 2 dummy variables indicating SBP tertiles, the product of LDLM and these 2 SBP dummy variables, and the covariates. The dummy variables and the linear regression were constructed as depicted in Table 1.

For a comprehensive exploration of the interactions between different kinds of blood pressures and lipids, similar analyses were conducted with serum total, LDL-C, and HDL cholesterol and with SBP as well as diastolic blood pressure (DBP). No interactions of these relations with sex were detected, so interactions with sex were not included.

Results

Descriptive statistics for the cohort by sex and tertiles of SBP are summarized in Table 2. Because of stratified sampling, the prevalence of current smokers and Hispanics was approximately twice that in the employee population from which the cohort was sampled. Note that the high SBP tertile group included a greater proportion of persons with diabetes and a larger percentage of subjects who were prescribed medication for treatment of hypertension or hypercholesterolemia.

The Figure depicts the relations between IMT and LDL-C quintiles within SBP tertile groups. Adjusted IMT had a monotonous relationship with SBP (P<0.001 for high versus middle SBP groups or high versus low SBP groups, P<0.005 for

<table>
<thead>
<tr>
<th>SBP Tertile</th>
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<tr>
<td>Low</td>
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The regression equation to estimate the main effects and interactions was specified as IMT\(=\alpha+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_4X_4X_5+\beta_5LDLM+\beta_6LDLM+(\text{covariates})+\epsilon\) (residuals), where \(\beta_3\) is the slope of IMT on LDLM in the high SBP group, \(\beta_2\) is the difference between the slope of IMT on LDLM in the high and low SBP groups, and \(\beta_6\) is the difference between the slope of IMT on LDLM in high and middle SBP groups.
middle versus low SBP groups). IMT was 0.692±0.007 mm, 0.657±0.006 mm, and 0.631±0.006 mm in the high, middle, and low SBP groups, respectively. However, this monotonic relation did not hold when LDL-C was low. The adjusted IMT in the lower two LDL-C quintiles (LDL-C<3.42 mmol/L) of the high SBP group was 0.659±0.009 mm; it was no longer statistically different from the adjusted IMT of the middle SBP group (P=0.8).

It is clear from the Figure that there was no significant linear trend in IMT across LDL-C quintiles in the low (β=−0.004±0.009, where β values are IMT [mm]/LDL-C [mmol/L]; P=0.64) and middle (β=−0.006±0.008, P=0.39) SBP tertile groups. However, there was an upward trend (β=0.025±0.008, P=0.002) in IMT with increasing LDL-C in the high SBP tertile group. The slope in the high SBP group was significantly greater than the slope in the middle (P=0.004) and low (P=0.014) SBP groups. The findings were comparable when total cholesterol (rather than LDL-C) was used in the previous analysis. IMT was significantly related to total cholesterol in only the high SBP tertile group (β=0.020±0.008, P=0.011). These slopes were not significant in the middle (β=−0.004±0.007, P=0.56) or low (β=−0.006±0.008, P=0.75) SBP tertile groups, and the interaction terms were significant between high SBP tertile and the middle (P=0.02) and low (P=0.02) SBP tertiles.

The linear relationships between HDL cholesterol and IMT were not significant in low (β=0.008±0.019, P=0.68), middle (β=−0.012±0.023, P=0.60), or high (β=−0.019±0.024, P=0.40) SBP tertile groups. None of the linear slopes were significantly different from each other.

Similar analysis of LDL-C and DBP yielded results in the same direction. When subjects were stratified into 3 groups based on BP tertile (low [67 to 85 mm Hg], middle [86 to 92 mm Hg], and high [93 to 128 mm Hg]), IMT was shown to be significantly related with LDL-C in the high BP tertile group (β=0.022±0.008, P=0.008) but not in the middle (β=0.012±0.008, P=0.13) or low (β=−0.008±0.009, P=0.36) DBP tertile group. The difference in the linear slopes was statistically significant between the high and low DBP tertile groups (P=0.01) and was moderately significant between the high and middle DBP tertile groups (P=0.09).

Similar analyses were performed on the subset of 413 subjects without diabetes or treatment for hypertension or
The finding that IMT is more strongly related to LDL-C under conditions of elevated SBP is consistent with predictions from the response-to-injury model of atherogenesis. Elevated blood pressure induces modifications to the endothelium that may establish the susceptibility of the artery wall to LDL-C-mediated atherosclerosis.

Similar analyses with total cholesterol or DBP yielded a comparable pattern of associations, but the magnitude of the interactions was reduced. Considering the close correlation between total cholesterol and LDL (r = 0.68, P = 0.0001) and between SBP and DBP (r = 0.93, P = 0.0001), these findings may indicate that LDL-C and SBP are the more direct measures of the factors that interact to impact arterial wall thickening due to atherosclerosis.

There are additional aspects of the findings with implications for the pathophysiology of IMT. As shown in the Figure and Table 2, IMT increased substantially, with an increase in SBP from the bottom to middle tertile, but IMT was not positively related to LDL-C in the middle SBP tertile group. One plausible explanation for this pattern is that the thickening that occurred in the middle SBP tertile is adaptive thickening of the intima, which may be dampened such that eosinophil infiltration, an inflammatory response with stimulation of growth factors, proliferation of smooth muscle cells, and lipid accumulation in the intima occurs in the high blood pressure tertile group, in which endothelial damage is more likely to initiate atherogenesis. In addition to inducing the damage that initiates atherogenesis, elevated blood pressure may also accelerate lipid deposition through the increased diffusion of lipoproteins into the subendothelial space. The combination of elevated serum LDL-C concentration and SBP may thus operate synergistically to produce the thickest intima-media complex.

When LDL-C was low, it was found that IMT increases between the low and middle tertiles of SBP, but there is no comparable increase between the middle and high SBP groups. This suggests that high blood pressure alone, in the absence of elevated LDL-C, is insufficient to induce mal-adaptive (atherosclerotic) intimal thickening beyond the adaptive thickening observed in the middle SBP tertile. This interpretation is supported by findings from animal models with induced hypertension. It is also consistent with the observation that in hypertensive patients with low cholesterol levels, left ventricular hypertrophy is common, but coronary artery disease is not. The escalating inflammatory cascade that characterizes the atherogenic response to blood pressure damage may require elevated LDL-C (or some other mediator) to accelerate the process. With low LDL-C, the cascade of inflammatory response to elevated blood pressure (involving cytokines, growth factors, monocyte colony-stimulating factor, and modified LDL-C) may be dampened such that repair is achieved without self-perpetuation. Thus, the adaptive thickening of the intima may reach a maximum in the middle SBP tertile, and further intimal thickening is achieved only with LDL-C-mediated atherosclerosis.

This response-to-injury explanation of our findings is indirectly supported by a comparison of findings from cross-sectional and longitudinal studies of blood pressure and IMT. In cross-sectional studies, the relationship between SBP and IMT is generally monotonically positive. However, in the few published longitudinal studies, studies found no relationship between baseline SBP and subsequent change in IMT. Interestingly, the other 2 studies found the association among control groups from lipid-lowering trials in which hypercholesterolemic subjects were recruited. For instance, in the Kuopio Atherosclerosis Prevention Study (KAPS), one of the subjects’ recruitment criteria was that LDL-C was consistently >4 mmol/L. These findings are explained by our model inasmuch as only those persons with elevated blood pressure (or some other source of injury) and elevated LDL-C would be expected to show atherosclerotic progression. This pattern of findings from cross-sectional and longitudinal studies is consistent with a process in which elevated blood pressure leads to adaptive wall thickening that reaches an equilibrium with the demands of elevated pressure (rather than continued thickening, as in atherosclerosis). Subsequently, the damaged endothelium induced by the increased pressure is subject to atherosclerosis if LDL-C is retained in the artery wall. A finding from an intervention study that supports the response-to-injury interpretation of our results is the regression of carotid artery IMT in one lipid-lowering trial. In the Asymptomatic Carotid Artery Plaque Study (ACAPS),
among subjects selected for elevated LDL-C (60th to 90th percentiles) and carotid wall thickening, the lovastatin intervention effect in the hypertensive patients was found to be larger than in the nonhypertensive patients receiving lovastatin.\textsuperscript{15} Compared with wall thickening in the nonhypertensive group, wall thickening in the participants with combined elevated blood pressure and LDL-C was more likely due to progressing atherosclerosis. Given the cross-sectional findings reported in the present study and the observational nature of the other studies cited, there are clearly alternative explanations of an interaction between SBP and LDL-C as they relate to carotid IMT. The interaction could arise because of a synergism of the 2 factors that does not involve the temporal sequence inherent in the response-to-injury model or that of pressure-adaptive wall thickening. Elevated blood pressure may, for example, increase the diffusion of LDL-C into the subendothelial space\textsuperscript{23,24} or prolong the retention of LDL-C in the intima.\textsuperscript{33} Elevated blood pressure could also tend to promote lesions initiated by elevated LDL-C. Given that SBP and LDL-C tend to be correlated,\textsuperscript{35} it is also plausible that the interaction between them is due to each being determined by some other factor(s) that induces atherosclerosis. The apparent synergism would then actually be due to a third factor that is indicated by the presence of both risk factors.

Several studies describe a possible synergism of multiple risk factors to account for the existence of collagenous fibrous plaques in the aorta or coronary arteries.\textsuperscript{36} A study of 129 autopsied cases in Oslo (Holme et al\textsuperscript{37}) also revealed an interactive role of SBP and total serum cholesterol on raised lesions in the coronary arteries. It is of interest that this finding was in the opposite direction compared with our finding: Holme et al reported that the correlations between serum cholesterol and coronary lesions were 0.485 in the low SBP tertile, 0.353 in middle SBP tertile, and 0.185 in high SBP tertile. Another clinical study found that IMT in hypercholesterolemic hypertensives was not significantly thicker than IMT in normocholesterolemic hypertensives.\textsuperscript{38}

A synergism between SBP and LDL for atherosclerosis is not supported by findings from cohort studies with incident coronary disease or mortality as end points. For example, in the Honolulu Heart Program, serum cholesterol and SBP are additive (rather than synergistic) in logistic or probit models of coronary heart disease risk (J.H. Dwyer, D. Reed, unpublished data, 2000). Such risk regression models are the equivalent of the additive form (no interaction) of the linear model with a continuous outcome, such as carotid IMT. However, the absence of synergism between serum cholesterol or LDL-C and hypertension in cohort studies with coronary heart disease end points does not necessarily contradict the injury hypothesis. Event end points include atherogenic and thrombotic effects of factors, and elevated blood pressure may play a role in thrombotic events as well as an injury role in atherogenesis. An example of a potential thrombotic effect of hypertension is the adverse impact of elevated blood pressure on endothelium-dependent vasodilation and blood rheology, and both of these factors have been implicated as promoters of the conversion of atherosclerosis to atherothrombosis.\textsuperscript{39}

The complete absence of a positive association between carotid IMT and LDL-C in the lower blood pressure groups (see Figure) is puzzling. If elevated LDL-C is sufficient to cause endothelial damage and induce atherosclerosis,\textsuperscript{40} then a positive gradient in these groups would be expected. However, even if elevated LDL-C must be preceded by injury to promote atherosclerosis, we might expect that injuries to the arterial wall due to other factors (that are uncorrelated with LDL-C) would induce a positive association. Our findings therefore suggest that elevated blood pressure is the major source of arterial injury that results in susceptibility to LDL-C-induced atherosclerosis. It is also plausible that atherosclerosis due to LDL-induced injury develops at a later age and that such effects will become apparent as the cohort ages.

In summary, the finding of a cross-sectional interaction between SBP and LDL-C as they relate to carotid wall thickness in asymptomatic healthy people is consistent with predictions of the response-to-injury model of atherogenesis.\textsuperscript{2} Given that this finding has not been reported previously and that there are numerous alternative interpretations, replication and longitudinal investigations are needed to further investigate this issue.

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