Clinical Investigation and Reports

Interaction Between Soluble Thrombomodulin and Intercellular Adhesion Molecule-1 in Predicting Risk of Coronary Heart Disease

Kenneth K. Wu, MD, PhD; Nena Aleksic, PhD; Christie M. Ballantyne, MD; Chul Ahn, PhD; Harinder Juneja, MD; Eric Boerwinkle, PhD

Background—Results from previous ARIC (Atherosclerosis Risk In Communities) analyses indicate that soluble intercellular adhesive molecule-1 (sICAM) and soluble thrombomodulin (sTM) levels are associated with risk of coronary heart disease (CHD) in an opposite direction. A high sICAM level increases the risk of CHD, whereas a high level of sTM has a lower risk of CHD. It was unclear whether there was an interaction between sTM and sICAM.

Methods and Results—Using a nested case-cohort design, we measured sTM and sICAM in 317 incident CHD cases and 726 non-cases from the ARIC participants. Consistent with our previous reports, sICAM values in the upper versus the lower tertile increased the risk of CHD event by ≈2-fold (95% confidence interval [CI], 1.46 to 2.87) whereas sTM values in the lower versus the upper tertile increased CHD risk by ≈4-fold (95% CI, 2.80 to 5.74). Interaction between these 2 parameters was determined by weighted Cox proportional hazard regression. A significant interaction (P=0.038) was noted. Combinatorial analysis shows a significant increase in CHD risk ratio (RR) (4.66, 95% CI, 1.89 to 11.46) of the lower sTM/upper sICAM group versus the upper sTM/lower sICAM group. Individuals whose sTM values were in the upper tertile had a RR below 1, even when sICAM were in the upper tertile. The RR of lower tertile sTM was increased by sICAM in a tertile-dependent manner.

Conclusion—Weighted Cox proportional hazard analysis shows a significant interaction between sTM and sICAM in predicting risk of CHD event. Combinatorial analysis reveals that an upper tertile sICAM had a significant increase in the risk of a CHD event only when sTM was in the lower tertile. (Circulation. 2003;107:1729-1732.)

Key Words: glycoproteins ■ cell adhesion molecules ■ coronary disease ■ risk factors

Vascular endothelium separates the subendothelial tissue from blood constituents and maintains vascular integrity and vascular tone by expressing vasoprotective and thromboreistant molecules. Thrombomodulin (TM) is one of the important vasoprotective molecules.1 It is a transmembrane protein expressed constitutively primarily in endothelial cells. It has a large extracellular region comprising a thrombin binding site.2,3 On binding to this site, thrombin alters its conformation and becomes active in converting protein C to activated protein C. Activated protein C digests activated coagulation factor V and VIII, thereby reducing the prothrombotic activity.1–3 TM-bound thrombin is internalized and degraded in endothelial cells. Thus, TM possesses several properties to protect arterial walls and reduce thrombotic tendency. The extracellular region of TM is cleaved constitutively into several fragments collectively called soluble TM (sTM). We have recently shown in a prospective follow-up of ARIC (Atherosclerosis Risk In Communities) healthy participants that individuals whose sTM levels are in the highest quintile have a significant reduction in risk of coronary heart disease (CHD) when compared with those in the lowest quintile.4 These results suggest that the sTM level in healthy subjects may reflect the level of intact TM expression on endothelial cells and that a high TM level is protective against coronary thrombosis. In contrast, intercellular adhesion molecule-1 (ICAM-1) is expressed on endothelial surface after inflammatory stimulation, and the extracellular region of ICAM-1 is cleaved which circulates as soluble ICAM-1 (sICAM).5 sICAM is considered as an important marker of inflammation. Results from the ARIC prospective study have shown that individuals whose sICAM levels are in the highest quartile have significantly increased CHD risk when compared with those in the lowest quartile.6

Thus, a high level of sTM signifies protection and has a lower risk of CHD, whereas a high sICAM level signifies an inflammatory state and increases risk of CHD. We postulated...
that sTM interacts with sICAM in predicting CHD risk. To test this hypothesis, we analyzed the risk ratio of sTM and sICAM in ARIC CHD cases and random reference cohort by a combinatorial approach.

Methods

Study Population

The ARIC study recruited a population-based cohort of men and women 45 to 64 years of age from 4 US communities in 1987 through 1988. A total of 15 792 participants completed a home interview and clinic examination. The participants were re-examined on a 3-year cycle; 93% of the initial cohort were re-examined in 1990 to 1992, 86% in 1993 to 1995, and 80% in 1996 to 1998 by identical procedures. These participants have been prospectively followed for development of CHD and other vascular events since enrollment. ARIC followed the cohort and ascertained CHD events using standardized methods described previously. We defined CHD incidence as (1) a definite or probable myocardial infarction (MI); (2) a silent MI; (3) definite CHD death; or (4) a coronary revascularization. A random sample of the entire cohort was selected to serve as reference by a procedure previously described. Participants were excluded if they were neither white nor black, had prevalent CHD at baseline, or had a history of stroke or transient ischemic attacks. For the present study, we included CHD events that occurred between the initial visit (1987 to 1989) and December 31, 1996. The mean follow-up period was 7.9 years. The proportion of the entire cohort lost to follow-up was 13.1%. After exclusions, the final sample contained 317 CHD cases and a reference cohort of 770, 44 of whom were also cases. After subtracting cases from the reference cohort, there were 726 random cohort samples that were designated as non-cases. Blood samples for sTM and sICAM measurements were collected during the first visit (1987 to 1989) before onset of CHD events.

Baseline Measurements

Blood pressure, anthropometry, carotid sonography, cigarette smoking, and other lifestyle parameters were determined during the first visit (1987 to 1989) by standardized procedures described previously. Venous blood was collected during the first visit according to standardized venipuncture and processing procedures. The processed plasma and serum samples were stored at −80°C until assay. sTM and sICAM levels in plasma were measured by enzyme immunosassays as described previously. Other coagulation and lipid parameters were measured by standardized methods, which have been described previously. The laboratory intra-assay coefficients of variation (CV) for sTM and sICAM were 6.0% and 4.4%, respectively, and the inter-assay CVs were 8.2% and 7.4%, respectively.

Data Analysis

We used a case-cohort design for this analysis. Plasma sTM and sICAM were determined in all 317 incident CHD cases and 726 non-cases. We defined 8 strata for sampling the cohort, as previously described. To account for the stratified sampling design in analyses, we weighted each observation with each stratum by the inverse sampling fraction for that stratum, thereby recreating the original frequency distribution of the strata in the entire cohort. We first used ANCOVA to compute age-, race-, and sex-adjusted mean levels of sICAM and sTM for CHD cases and non-cases after appropriate weighing for the stratified sampling design. We also used ANCOVA to compute age-, race-, and sex-adjusted mean or percentage values of study variables according to the upper and lower tertiles of sTM and sICAM in the cohort sample after appropriate weighting for the stratified sampling design. We computed the risk ratios and 95% confidence intervals (CI) for the time to the development of CHD in relation to sTM and sICAM using a weighted proportional hazard regression previously described by Barlow. We examined if there was a significant interaction between sTM and sICAM by weighted Cox proportional hazard regression. We trichotomized the sTM and sICAM levels to determine the risk ratios of upper tertile sTM/upper tertile sICAM (u T/u I) versus 8 other groups: u T/l I, u T/middle tertile sICAM (m I), l T/u I, l T/middle tertile sICAM (m I), m T/l I, m T/middle tertile sICAM (m I) and l T/l I. We examined if the baseline characteristics were significantly different between the reference and 8 comparison groups by using Bonferroni correction to adjust for the effect of multiple-group comparisons. A probability value less than 0.0062 (=0.05/8) was considered significant.

Results

The age-, race-, and sex-adjusted mean value of sICAM was significantly higher in cases than in non-cases (279 versus 239 ng/mL, P<0.0001). The adjusted mean value of sTM was lower in cases than in non-cases, but the difference did not reach statistical significance (37.6 versus 41.5 ng/mL, P=0.068). These results are consistent with our earlier analysis of a smaller number of cases. We next analyzed the relative CHD risk of upper versus lower tertile sICAM or lower versus upper tertile sTM individually with adjustment made for age, sex, race, and conventional cardiovascular risk factors. The risk ratio of a CHD event for participants whose sICAM values were at the upper tertile was 2-fold higher than those whose sICAM values were at the lower tertile (Table 1). By contrast, participants whose sTM values were at the lower tertile had a 4-fold higher increased risk of CHD than those whose sTM values were at the upper tertile (Table 1). These results revealed an association of these 2 endothelial cell markers with CHD risk in opposite direction and suggested a potential interaction between them. Weighted Cox proportional hazard analysis revealed a significant interaction between sTM and sICAM in influencing risk of CHD (P=0.038). To characterize this interaction, we analyzed the risk ratio according to combined sTM and sICAM tertiles. There are 9 groups as shown in Table 2. Group 1 (u T/l I) was chosen as the reference group as theoretically it has the lowest risk. Number of subjects, age, race, sex, and conventional risk factor profile of each group are shown in Table 2. When compared with the reference group, group 7 (l T/l I) was significantly younger; group 2 (u T/m I) and group 3 (u T/u I) had a significantly lower percentage of blacks; group 7 has a lower percentage of men; group 6 (m T/u I) and group 9 (l T/u I) had a significantly higher level of cigarette smoking per
sTM values. sTM in the upper tertile significantly reduces the risk of a CHD event even in the presence of sICAM in the upper tertile. As shown in the Figure, the RRs of the u T/u I and u T/m I groups were below 1, and for reasons unclear at the present time, the RR of u T/m I was significantly lower than that of the reference group (u T//I). Risk assessment by sICAM analysis alone would have labeled the u T/u I group as having a 2-fold increase in CHD risk. In fact, a high sICAM value is associated with an increased CHD risk only when the sTM value is low.

Discussion

A major finding of this study is that there is a significant interaction between sTM and sICAM in predicting the risk of a CHD event in middle-aged men and women. By a combinatorial analysis, our results provide new information regarding the CHD risk assessment using these 2 soluble endothelial markers. Association of these 2 markers with CHD risk is greatly influenced by the sTM values. sTM in the upper tertile year; group 7 and group 8 (l T/m I) had a significantly lower fibrinogen level; and group 6 (m T/u I) had a significantly higher white blood cell (WBC) count (Table 2). These differences were taken into consideration when we analyzed the risk ratio of CHD events for each group.

After adjusting for age, sex, race, and several conventional risk factors (total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, triglyceride, systolic blood pressure, hypertension, diabetes mellitus, cigarette smoking, fibrinogen, and WBC), the risk ratio (RR) for the time to develop a CHD event of the l T/u I group was significantly higher (RR = 4.66, 95% CI, 1.89, 11.46), whereas the RR of the u T/m I group was significantly lower (RR = 0.37, 95% CI, 0.14, 0.95) than that of the reference group (u T//I). The RR of the u T/u I group was also below 1, but the difference was not statistically significant (Figure 1). The 3 groups with sTM values at the lower tertile exhibited a graded increase in RR according to the tertile of sICAM: 4.66 for the l T/u I, 2.50 for the l T/m I, and 1.39 for the l T//I group (Figure 1). The 3 groups with sICAM values at the upper tertile also exhibited a sTM tertile-dependent increase in RR. The RR of the m T/u I group (2.50) was between that of l T/u I and a T/u I (Figure 1). The remaining 2 groups (m T//I and m T/m I) had a RR less than 1 which, however, was not significantly different from the reference.

**TABLE 2.** Characteristics of 9 Groups of Subjects Based on sTM and sICAM-1 Tertiles

<table>
<thead>
<tr>
<th>Group</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
<th>Group 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>u T/u</td>
<td>u T/m</td>
<td>u T/u</td>
<td>m T/m</td>
<td>m T/u</td>
<td>m T/u</td>
<td>I T/u</td>
<td>I T/m</td>
<td>I T/u</td>
<td>I T/m</td>
</tr>
<tr>
<td>(n=82)</td>
<td>(n=96)</td>
<td>(n=78)</td>
<td>(n=88)</td>
<td>(n=83)</td>
<td>(n=86)</td>
<td>(n=87)</td>
<td>(n=78)</td>
<td>(n=92)</td>
<td></td>
</tr>
<tr>
<td>Age at first visit, y</td>
<td>54.2</td>
<td>56.5</td>
<td>55.3</td>
<td>53.7</td>
<td>53.7</td>
<td>53.0</td>
<td>51.9</td>
<td>53.1</td>
<td>54.5</td>
</tr>
<tr>
<td>Blacks, %</td>
<td>32.4</td>
<td>7.4†</td>
<td>6.9†</td>
<td>36.4</td>
<td>15.5</td>
<td>28.0</td>
<td>24.3</td>
<td>26.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Men, %</td>
<td>56.6</td>
<td>47.7</td>
<td>45.3</td>
<td>56.5</td>
<td>50.7</td>
<td>51.6</td>
<td>46.1</td>
<td>30.0</td>
<td>32.3</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>209</td>
<td>216</td>
<td>222</td>
<td>215</td>
<td>209</td>
<td>214</td>
<td>216</td>
<td>222</td>
<td>202</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>52.4</td>
<td>55.6</td>
<td>49.4</td>
<td>55.3</td>
<td>50.6</td>
<td>50.6</td>
<td>53.7</td>
<td>56.8</td>
<td>57.8</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>127</td>
<td>127</td>
<td>159</td>
<td>107</td>
<td>123</td>
<td>127</td>
<td>128</td>
<td>328</td>
<td>282</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>127</td>
<td>119</td>
<td>125</td>
<td>120</td>
<td>120</td>
<td>120</td>
<td>118</td>
<td>117</td>
<td>121</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>43.1</td>
<td>28.1</td>
<td>36.6</td>
<td>25.9</td>
<td>29.1</td>
<td>29.0</td>
<td>20.2</td>
<td>16.3</td>
<td>26.6</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>3.1</td>
<td>2.2</td>
<td>5.3</td>
<td>0.6</td>
<td>6.0</td>
<td>5.0</td>
<td>1.3</td>
<td>3.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Cigarettes per year</td>
<td>136</td>
<td>276</td>
<td>289</td>
<td>285</td>
<td>281</td>
<td>346§</td>
<td>207</td>
<td>279</td>
<td>475§</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>302</td>
<td>300</td>
<td>304</td>
<td>295</td>
<td>297</td>
<td>328</td>
<td>346§</td>
<td>207</td>
<td>279</td>
</tr>
<tr>
<td>WBC, count/μL × 10^9</td>
<td>5.7</td>
<td>6.1</td>
<td>6.4</td>
<td>5.6</td>
<td>6.3</td>
<td>6.7§</td>
<td>5.6</td>
<td>6.1</td>
<td>6.6</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein; BP, blood pressure.

*Age was significantly younger than that of the reference group (P<0.001).
†Percentage of blacks was lower than that of the reference group (P<0.0003).
‡Percentage of men was significantly lower than that of the reference group (P<0.005).
§Cigarettes/year were significantly higher than that of the reference group (P<0.006).
¶White blood cell (WBC) count was higher than that of the reference group (P<0.003).
Interestingly, sTM and sICAM exert a dose-dependent effect on the risk ratio. The tertile-dependent response was most apparent in the 3 groups with sTM values in the lower tertile; the RR's for the L/T/L, L/T/m, and L/T/u groups were 1.39, 2.50, and 4.66, respectively. Three groups with sICAM values in the upper tertile also exhibited a tertile-related effect; the RR for the u/T/l, m/T/l, and L/T/u groups were 0.78, 2.35, and 4.66, respectively. Taken together, these results support the proposal that sTM may reflect the level of endothelial TM, and a high TM level may protect vascular wall from inflammatory insults. Recent experimental data have provided evidence for an anti-inflammatory action of TM.44

The plasma level of sTM in healthy ARIC participants could be influenced by the level of endothelial TM expression, the rate of its basal cleavage, and changes in TM expression and cleavage caused by subclinical inflammation. Inflammation has been recognized as a major component in the pathophysiology of atherothrombosis, and inflammatory markers are associated with an increased risk of CHD.15,16 Proinflammatory mediators have been reported to suppress endothelial TM expression and thus may reduce the level of sTM.17,18 On the other hand, proinflammatory mediators induce ICAM-1 expression, increase cleavage of ICAM-1, and increase sICAM levels.19,20 Thus, a high sICAM and a low sTM level could represent a highly active inflammatory state and would be anticipated to have a high risk of CHD events. Our data support the role of inflammation in unstable plaque and the consequent plaque rupture and thrombosis.21

Results from our study open a new avenue for assessing risk of CHD. Conventional risk assessment by biochemical markers tends to evaluate the risk ratio of a single marker and, in the case of multiple markers, tends to be random without a clear pathophysiological basis. Our results show that the CHD risk may be more clearly defined by coupling two markers with opposite pathophysiological indications than by each individual marker. Because the occurrence of an arterial thrombotic event is determined by a balance between prothrombotic and anti-thrombotic factors, the combinatorial analysis may be extended to include additional factors. For example, results from ARIC studies have shown an association of several procoagulant factors and fibrinolytic factors with the risk of CHD events.10,22 We have carried out a preliminary combinatorial analysis to determine the interaction of sTM with prothrombotic factors, and the results show a positive interaction of sTM with fibrinogen, factor VIII, and von Willebrand factor levels. Work is in progress to characterize the risk by combinatorial analysis.

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