Circulating Human Heat Shock Protein 60 in the Blood of Healthy Teenagers: A Novel Determinant of Endothelial Dysfunction and Early Vascular Injury?

To the Editor:

Damage and dysfunction of vascular endothelial cells plays a critical role in the initiation and progression of atherosclerosis, with inflammation implicated as a key mediator of endothelial dysfunction and early structural arterial disease in childhood. However, the specific molecular triggers of local vascular inflammation are less clear. One potential mediator relevant to endothelial dysfunction and atherogenesis is heat-shock protein 60 (HSP60). Intracellular soluble HSP60 (sHSP60) has also been identified in the blood of healthy adults, and sHSP60 levels correlate with inflammation implicated as a key mediator of endothelial dysfunction and early carotid disease implicating HSP60 in atherogenesis.

To explore a potential role for HSP60 in early atherogenesis we determined whether sHSP60 was detectable in the blood of healthy adolescents and examined the relationship with clinical measures of vascular function and structural arterial pathophysiology. Young study populations minimize potentially confounding influences of long-term modest risk factor exposure, increasing the ability to evaluate the clinical impact of novel putative atherogenic determinants. We studied 294 healthy adolescents (aged 13 to 16 years). All subjects were nonsmokers and free from hypertension, dyslipidemia, and diabetes mellitus. Anthropometric, demographic, and metabolic characteristics of this cohort have been published previously. All subjects underwent noninvasive assessment of flow-mediated vasodilatation (FMD) and distensibility of the brachial artery using high resolution ultrasound to determine endothelial function and arterial stiffness, respectively. Serum sHSP60 was assayed using a highly specific ELISA according to the method described by Lewthwaite.

Primary comparisons were made between adolescents with and without detectable sHSP60. Multiple linear regression analysis was used to assess associations between vascular measures and sHSP60 expression with adjustment for potential confounding factors (age, sex, BMI, arterial diameter, CRP, and lipids).

Serum from 256 subjects was available for analysis. sHSP60 was detectable in 60 subjects over a wide range of values (Figure, a). HDL cholesterol (1.27 versus 1.16 mmol/L, P = 0.03) levels in those born at term were slightly higher, but other variables (total cholesterol CRP, blood pressure, BMI, age, and gender) were similar in subjects with and without detectable sHSP60. There was no association between age and log(sHSP60) in those with detectable levels (r = 0.07, P = 0.3), and expression of sHSP60 was similar in subjects born at term versus preterm (P = 0.9); levels in those born at term and preterm with detectable sHSP60 were 770 [5937] versus 874 [1819] ng/mL (median [interquartile range]) respectively. There was no association between any other variable and sHSP60 level, although a weak positive correlation was observed between HDL and log(sHSP60) levels (r = 0.116, P = 0.06). Of note, no association was observed between CRP and log(sHSP60) levels (r = −0.029, P = 0.648).

FMD was significantly lower in adolescents positive for sHSP60 compared with those who were negative (0.198 ± 0.010 mm versus 0.228 ± 0.008 mm, mean ± SEM, P = 0.02; Figure, b). This relationship was similar with and without adjustment for baseline artery diameter (P = 0.02). The association between sHSP60 expression and impaired FMD also remained after further adjustment for multiple potential confounding factors (β = −0.133, P = 0.03). Baseline diameter (β = −0.219, P = 0.004) and age (β = 0.165, P = 0.009) were also associated with FMD in this model. FMD was not related to blood pressure, BMI z score, or to CRP, glucose, insulin, or lipid levels. We also found that FMD expressed as a percentage change in diameter was lower in subjects expressing sHSP60 (P = 0.02, adjusted for baseline diameter), even after multivariable adjustment for confounders including CRP (β = −0.123, P = 0.03).

Of subjects with detectable sHSP60, those in the higher quartile of log(sHSP60) distribution had impaired FMD compared with those in the lower quartile (P = 0.05). Brachial artery diameter (P = 0.2) and log(arterial distensibility (4.75 ± 0.38 versus 4.75 ± 0.04, 7 = 0.7) were similar in subjects with and without detectable sHSP60. No relationship was observed between log(sHSP60) levels and distensibility (P = 0.5).

This study is the first to demonstrate circulating sHSP60 in children. We observed that teenagers with detectable sHSP60 had significantly lower FMD than the remainder who lacked this stress protein in their blood, and found a subtle relationship between log(sHSP60) levels and FMD. Despite the relatively small number of children with detectable sHSP60 in their blood, our data reflect the previously described relationship between sHSP60 and structural arterial disease in adults. FMD was not associated with any conventional risk factors, which was not unexpected in these healthy children without disturbances in these parameters. Only HDL cholesterol was higher in the group with sHSP60 compared with the remainder of the population. Other relevant risk factors were similar in both groups. Notably, neither FMD nor sHSP60 were associated with CRP levels, making it unlikely that nonspecific low-grade inflammatory changes are responsible for the relationship between sHSP60 and endothelial dysfunction.

We did not perform assessment of endothelium-independent vascular function with GTN in our study, so we cannot selectively localize the functional deficit to the endothelium. However, smooth muscle dysfunction is often associated with atherosclerosis, typically occurring at a later stage in the disease process than endothelial dysfunction. Thus our findings are likely to reflect relevant pathophysiology. Considered together, these findings suggest that sHSP60 may contribute to the initiation of arterial disease in early life. Activation of cells involved in atherogenesis, including leukocytes, smooth muscle, and endothelial cells, via HSP60-mediated CD14/TLR4 binding and activation of the p38 MAPK pathway could be a putative mechanism.

Despite the strong relationship between sHSP60 and FMD, we found no evidence of an association between sHSP60 and brachial artery distensibility, a measure of arterial stiffness that typically represents more established arterial pathophysiology. Although this is at odds with adult data demonstrating a relationship between sHSP60 and carotid atherosclerosis, endothelial dysfunction typically precedes the development of structural changes in the vessel wall. Thus, our cross-sectional analysis of this healthy cohort likely represents a snapshot of the very early disease process, in which mild vasomotor dysfunction related to sHSP60 has not yet led to established structural disease. A cross-sectional study design such as ours cannot confirm causal relationships, and it is possible that elevated sHSP60 may be a consequence rather than a cause of early atherogenesis. Although it is difficult to extrapolate the long-term clinical significance of these preclinical findings, the difference in FMD between our subjects with and without sHSP60 is equivalent to exposure to a single conventional risk factor for atherosclerosis. Ongoing follow-up of this and other young cohorts will allow us to evaluate prospectively the determinants and longer-term functional and structural vascular implications of circulating sHSP60 in humans.

In conclusion, we have shown for the first time that sHSP60 is detectable in the serum of approximately 25% of healthy children. Furthermore we have also shown that endothelium-dependent vasodilator function was impaired in children with compared to those without detectable sHSP60, even after adjustment for potential confounding variables including CRP. These findings suggest that sHSP60, or factors that stimulate the expression and systemic release of sHSP60, may contribute to the initiation of arterial disease in early life.
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