RELATIONSHIPS BETWEEN AUTOANTIBODIES AGAINST N-HOMOCYSTEINYLATED PROTEINS, HOMOCYSTEINE, AND STROKE IN HUMANS

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Introduction

Despite advances in our understanding of cardiovascular disease, traditional risk factors such as hypertension, smoking, diabetes, and hyperlipidemia do not accurately predict cardiovascular events. Clinical studies have shown that plasma total homocysteine (tHcy) is a risk factor for ischemic heart disease and stroke in humans and predicts mortality independently of traditional risk factors in patients with coronary artery disease [1,2]. Possible cellular mechanisms by which Hcy may contribute to vascular disease include unfolded protein response, oxidative stress, and the induction of pro-inflammatory factors [3]. A proposed molecular mechanism underlying Hcy toxicity in humans involves metabolic conversion of Hcy to Hcy-thiolactone, which subsequently modifies proteins causing their damage and inducing a variety of pathophysiological effects [4].

Multiple Molecular Forms of Hcy in Humans

Hcy exists in circulation in several molecular forms, including free reduced Hcy, homocysteine, mixed disulfides with albumin (S-Hcy-protein) and cysteine (Hcy-S-S-Cys) [5]. The sum of these Hcy species is called “total” Hcy, tHcy. S-Hcy-protein comprises ~80% of tHcy [5]. Recently, two novel Hcy metabolites, Hcy-thiolactone [6,7] and protein N-linked Hcy (N-Hcy-protein) [7-9], have been discovered in human blood and mechanisms of their biosynthesis elucidated [4]. The level of N-linked Hcy in an individual blood protein is proportional to the protein’s abundance [8] and correlates with the protein’s reactivity toward Hcy-thiolactone [10]. N-Hcy-protein constitutes a major pool of Hcy in normal human blood (~15.6 µM), with N-linked Hcy in hemoglobin and albumin comprising 75% and 22%, respectively, of this pool [8].

Which Molecular Forms of Hcy are Harmful?

Hcy-thiolactone and free reduced Hcy, which comprise ≤ 1.4 % [6] and 1-2 % [5] of plasma tHcy, respectively, appear to be harmful. For example, free reduced Hcy is associated with endothelial dysfunction whereas much more abundant disulfide forms of Hcy are not [11]. Free reduced Hcy is the only form of Hcy that can be metabolized directly to Hcy-thiolactone [4,12-15]. Patchy desquamation of vascular endothelium and arterial thrombosis in response to chronic infusions of Hcy-thiolactone occur in baboons used as an early model of clinical homocystinuria.
Recent studies show that Hcy-thiolactone is more potent than Hcy in inducing death in cultured human endothelial cells [17]. Hcy-thiolactone is harmful because of its ability to modify proteins [4,7,9,10,15]. Protein N-linked Hcy is also detrimental because it can induce cell death [18] and immune response [19,20].

**Mechanism of Nε-Hcy-Lys-protein Synthesis**

Hcy-thiolactone forms in human body in a reaction catalyzed by methionyl-tRNA synthetase (MetRS) according to equation immediately below [12].

\[
\text{MetRS} + \text{Hcy} + \text{ATP} \rightleftharpoons \text{MetRS} \cdot \text{Hcy} \cong \text{AMP} \Rightarrow \text{Hcy-thiolactone} + \text{MetRS}
\]

Hcy-thiolactone subsequently forms Nε-Hcy-Lys-protein adducts, in which Hcy is N-linked to the ε-amino group of protein lysine residues as seen in the following equation [4,9,10,13-15].

**Nε-Hcy-Lys-proteins are Functionally Damaged**

Nε-Hcy-Lys-proteins are functionally different from native proteins. For instance, Nε-Hcy-Lys-cytochrome c aggregates due to intermolecular disulfide bond formation [10]. Nε-Hcy-Lys-hemoglobin, in contrast to native hemoglobin, is susceptible to further irreversible damage by oxidation [4]. Nε-Hcy-Lys-albumin exhibits greater susceptibility to proteolytic degradation than unmodified albumin [4,9]. Modification with Hcy-thiolactone decreases activity of paraoxonase carried on HDL, which renders it less protective against oxidative damage [21]. Nε-Hcy-Lys-LDL induces oxidative damage and cell death in cultured endothelial cells [18].

**Anti-Nε-Hcy-Lys-protein Autoantibodies Occur in Humans**

We hypothesized that Nε-Hcy-Lys-proteins would be recognized as neo-self antigens and induce autoimmune response. We found that each human serum tested showed some titer of IgG [19] and IgM auto-antibody (unpublished) against Nε-Hcy-Lys-proteins. The antigen specificity of the human IgG auto-antibody, tested with structural analogues of Nε-Hcy-Lys epitope as competitors and with various Nε-Hcy-Lys-proteins as an antigen, is identical to that of a rabbit antibody raised against Nε-Hcy-Lys-protein [19,20]. The high specificity of the human auto-antibody is illustrated by our finding that Nε-acetyl-Nε-Hcy-Lys, in contrast to Nε-Hcy-Nε-acetyl-Lys, does not compete with the human IgG binding. Our data suggest that human IgG specifically recognizes Nε-Hcy-Lys epitope on Nε-Hcy-Lys-protein. Serum levels of anti-Nε-
Hcy-Lys-protein IgG are positively correlated with plasma tHcy, but not cysteine or methionine, levels [19].

Anti-$N\varepsilon$-Hcy-Lys-protein Autoantibodies in Stroke Patients

Significant differences in mean levels of anti-$N\varepsilon$-Hcy-Lys-protein IgG auto-antibody were found between the group of male patients with stroke and the group of healthy subjects [19]. Male stroke patients had higher serum levels of anti-$N\varepsilon$-Hcy-Lys-protein IgG than healthy controls. Male stroke patients had also higher levels of plasma tHcy than controls, consistent with earlier studies [1,2]. Plasma levels of tHcy and anti-$N\varepsilon$-Hcy-Lys-protein IgG auto-antibody in female stroke patients were similar to corresponding levels in female controls. There were no differences in plasma cysteine or methionine concentrations between stroke patients and controls both for males and females. Higher levels of anti-$N\varepsilon$-Hcy-Lys-protein autoantibodies are present in male, but not female, stroke patients, compared to healthy controls, most likely reflect higher levels of Hcy in male stroke patients.

Immune activation modulates atherogenesis [22,23]. Chief risk factors for atherogenesis, such as dyslipidemia and diabetes contribute to inflammatory conditions through lipid peroxidation, glycoxidation, and increased secretion of pro-inflammatory cytokines [22]. Increased plasma levels of inflammation markers, such as C-reactive protein, interleukin 6, serum amyloid A, interleukin 1 receptor antagonist, and soluble adhesion molecules, are independent predictors of coronary events [23]. Some antigens, such as modified artery wall proteins and oxidized or glycated LDL, causing immune activation have been identified. Our findings that anti-$N\varepsilon$-Hcy-Lys autoantibodies are correlated with tHcy and stroke, identify a novel neo-self antigen, $N\varepsilon$-Hcy-Lys-protein, and also suggest a mechanism by which Hcy contributes to immune activation in stroke [19].

Levels of anti-$N\varepsilon$-Hcy-Lys-protein autoantibodies [19] and $N$-Hcy-protein [8] vary considerably among human subjects and are correlated with plasma tHcy. Such correlations can be explained by direct mechanistic links between these Hcy-related species. Elevation in Hcy levels leads to inadvertent elevation in Hcy-thiolactone according to the first equation above, observed in cultured human endothelial cells [14] and in human plasma (unpublished). Hcy thiolactone mediates Hcy incorporation into proteins [4] and thus the formation of neo-self antigens, $N\varepsilon$-Hcy-Lys-protein, according to the second equation above. Raising levels of neo-self $N\varepsilon$-Hcy-Lys-protein subsequently trigger an immune response. Autoantibodies recognizing $N\varepsilon$-Hcy-Lys neo-epitope would react with $N\varepsilon$-Hcy-Lys-proteins in many tissues, possibly contributing to deleterious effects of hyperhomocysteinemia on many organs [1,2].

Anti-$N\varepsilon$-Hcy-Lys-protein autoantibodies could clear $N\varepsilon$-Hcy-Lys-proteins from circulation, which would be beneficial. However, if $N\varepsilon$-Hcy-Lys-proteins were present on endothelial cells, this could lead to the formation of antigen-antibody complexes on the surface of the vascular vessel. Endothelial cells coated with anti-$N\varepsilon$-Hcy-Lys-protein autoantibodies would be taken up avidly by the macrophage via the Fc receptor, resulting in injury to vascular surface. If the neo-self antigen $N\varepsilon$-Hcy-Lys, which initiates the injury, were present continuously, repeating attempts to repair the damaged vascular wall would lead to an atherosclerotic plaque.
In conclusion, our data suggest a novel mechanism by which Hcy elicits anti-\(\epsilon-N^2\)-Hcy-Lys-protein auto-antibody formation and links the extent of the antibody induction with stroke in humans.

References

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