Homocysteine, a Proinflammatory and Proatherosclerotic Factor

Role of Intracellular Reactive Oxygen Species

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In 1969, McCully first described the association of hyperhomocysteinemia and vascular disease in a patient with elevated levels of homocysteine in plasma and urine due to a rare genetic deficiency of homocysteine metabolism. A large number of clinical studies have indicated that mild hyperhomocysteinemia, which occurs in approximately 5% to 7% of the general population, is also associated with vascular disease including coronary artery, cerebrovascular, and peripheral arterial occlusive diseases. Elevated plasma levels of homocysteine are considered as an independent risk factor for atherothrombotic disease. Both experimental and clinical data suggest that elevated plasma concentrations of homocysteine might accelerate the development of atherosclerotic lesions to advanced atherosclerotic plaques, which are sites of endothelial erosion and rupture triggering the formation of occlusive mural thrombi and ischemic events.

Homocysteine is an intermediate sulfur-containing amino acid formed during the intracellular metabolism of methionine, an essential amino acid supplied by dietary proteins. Once homocysteine is formed, it may be recycled to methionine after remethylation by two different pathways. The first one involves methionine synthase, an enzyme that uses vitamin B12 (cobalamin) as an essential cofactor and N5-methyl-tetrahydrofolate as the methyl donor. The second pathway, which occurs exclusively in hepatic tissue, involves the enzyme betaine-homocysteine methyltransferase. Homocysteine may also be converted to cystathionine by cystathionine β-synthase, a vitamin B6-dependent enzyme, which is subsequently hydrolyzed to form cysteine by cystathionine γ-lyase. Cysteine, in turn, can be used to synthesize the antioxidant glutathione or be further metabolized to sulfate and excreted in the urine. Under physiological conditions, the formed homocysteine is predominantly remethylated to methionine by methionine synthase, whereas in conditions in which an excess of methionine is present, or cysteine synthesis is required, homocysteine enters the pathway leading to the formation of cysteine. Plasma levels of homocysteine refer to the total pool of homocysteine. There is very little free homocysteine since the vast majority of homocysteine is present either in a protein-bound form or as a dimer with itself or cysteine. Physiological plasma concentrations of homocysteine range from 5 to 15 μmol/L. Elevated levels of homocysteine concentrations are categorized as moderate (concentrations ranging from 16 to 30 μmol/L), intermediate (31 to 100 μmol/L), and severe (>100 μmol/L). Mild hyperhomocysteinemia can be the consequence of age, gender, and nutritional deficiencies in the vitamin cofactors, which are required for the metabolism of homocysteine (including vitamin B12 and vitamin B6, and in the cosubstrate folate), as well as a consequence of smoking, renal disease, hypothyroidism, several types of cancer, and in response to drugs like methotrexate, phenytoin, cyclosporine, and metformin. Severe hyperhomocysteinemia is rare among the general population. It is caused by genetic defects in the enzymes involved in homocysteine metabolism, the most common disorder being a deficiency of cystathionine β-synthase, resulting in marked elevations of plasma and urine homocysteine concentrations. Although hyperhomocysteinemia has been linked clinically to vascular disease, the molecular mechanism by which elevated plasma levels of homocysteine are related to the pathogenesis of atherothrombotic disease remains unclear.

In this issue of Circulation Research, Zeng and colleagues present a mechanism of potential clinical importance that might be involved in the progression of atherosclerotic lesions in patients with hyperhomocysteinemia. The authors report that homocysteine stimulates the expression and secretion of biologically active monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8), two major chemokines for leukocyte trafficking, in human monocytes. These findings extend previous ones indicating that homocysteine induces the expression of MCP-1 and IL-8 in human aortic endothelial cells and MCP-1 in human vascular smooth muscle cells. Moreover, examination of human atherosclerotic plaques has indicated a pronounced expression of both MCP-1 and IL-8, predominantly in macrophages. The present study also indicates that the stimulatory effect of homocysteine on MCP-1 and IL-8 expression and secretion in human monocytes is observed at concentrations as low as 10 μmol/L. Thus, mild hyperhomocysteinemia might provide an efficient stimulus for the accumulation of MCP-1 and IL-8 in the injured arterial wall, thereby...
promoting the localized accumulation of macrophages and contributing to proinflammatory and proatherosclerotic responses. Other plausible mechanisms of homocysteine-induced atherosclerosis include endothelial dysfunction, increased vascular smooth muscle cell proliferation and platelet activation, promotion of lipoprotein oxidation, enhanced coagulability, and increased synthesis of cholesterol in hepatocytes.13,14

The work of Zeng and colleagues7 also focused on the characterization of the signal transduction pathway(s) mediating the stimulatory effect of homocysteine on both MCP-1 and IL-8 expression in human monocytes. These investigations indicate a determinant role of oxidative stress in the stimulatory effect of homocysteine most likely via activation of the redox-sensitive transcription factor NF-κB. The oxidant response to homocysteine as assessed by the redox-sensitive fluorescent probe dichlorofluorescein was not explained by the extracellular auto-oxidation of homocysteine but rather due to the intracellular formation of reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and hydroxyl radicals. Moreover, the pharmacological characterization of the enzymatic source of ROS has suggested a major role for the NAD(P)H oxidase whereas xanthine oxidase, arachidonic acid metabolism, and the mitochondrial respiratory chain were not involved. However, a word of caution should be spoken, since this conclusion is reached solely on findings obtained with two unselective inhibitors of the NAD(P)H oxidase, diphenylethylenediamine and phenylarsine oxide. It remains to be determined whether more selective inhibitors of the NAD(P)H oxidase or the use of the p22phox antisense approach will lead to a similar conclusion. Nevertheless, the observations of Zeng and colleagues provide evidence of a novel link between a redox-sensitive proatherogenic response induced by homocysteine and the NAD(P)H oxidase. NAD(P)H oxidase is a multicomponent superoxide-generating enzyme, which is thought to play a major role in the pathogenesis of atherosclerosis.15 Although the molecular mechanism relating homocysteine-induced activation of human monocytes and the generation of ROS still remains unclear, a role for protein kinase C and calmodulin has been suggested by the authors based on the findings that pharmacological inhibition of these two pathways partially prevented the response to homocysteine.

One highly interesting aspect of the manuscript by Zeng et al7 is the suggestion that activators of peroxisome proliferator-activated receptor γ (PPARγ) might effectively prevent the proatherogenic response of monocytes to homocysteine. Indeed, both ciglitazone and troglitazone, two activators of PPARγ, strongly inhibited the homocysteine-induced production of MCP-1 and IL-8. The protective effect of both PPARγ activators appears to be due to their ability to attenuate the intracellular formation of ROS. Similar protective effects have been shown in previous studies and linked to the ability of PPARγ activators to reduce the expression of major NAD(P)H oxidase components such as p22phox and p47phox in human endothelial cells and p47phox in a human monocyte cell line.16,17 In addition, an upregulation of Cu2+-Zn2+-superoxide dismutase might also contribute to the protective effect in human endothelial cells.16 In summary, the work of Zeng and colleagues7 demonstrates that pathophysiological relevant concentrations of homocysteine elicit the expression and secretion of biologically active MCP-1 and IL-8 in human monocytes. The stimulatory effect of homocysteine is critically dependent on the formation of intracellular ROS and may involve the activation of the NAD(P)H oxidase. This study provides additional experimental evidence suggesting that mild hyperhomocysteinemia might promote atherogenesis by stimulating infiltration of leukocytes to sites of vascular injury. In addition, the potential protective effect of PPARγ activators might be due, at least in part, to the attenuation of the proatherogenic effect of homocysteine.

**References**

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