Mechanisms of homocysteine toxicity in humans

Review Article

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Summary. Homocysteine, a non-protein amino acid, is an important risk factor for ischemic heart disease and stroke in humans. This review provides an overview of homocysteine influence on endothelium function as well as on protein metabolism with a special respect to posttranslational modification of protein with homocysteine thiolactone. Homocysteine is a pro-thrombotic factor, vasodilation impairing agent, pro-inflammatory factor and endoplasmatic reticulum-stress inducer. Incorporation of Hcy into protein via disulfide or amide linkages (S-homocysteinylation or N-homocysteinylation) affects protein structure and function. Protein N-homocysteinylation causes cellular toxicity and elicits autoimmune response, which may contribute to atherogenesis.

Keywords: Homocysteine – Homocysteine thiolactone – Protein N-homocysteinylation – Toxicity – Autoantibodies – Protein S-homocysteinylation

Abbreviations: APC, activated protein C; BLH, bleomycin hydrolase; ER, endoplasmatic reticulum; HDL, high density lipoprotein; HTL, homocysteine thiolactone; LDL, low density lipoprotein; MetRS, methionyl-tRNA synthetase; MS, methionine synthase; PON, paraoxonase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; TPA, tissue plasminogen activator; UPR, unfolded protein response; VEGF, vascular endothelial growth factor

Introduction

Hyperhomocysteinemia has been recognized as a risk factor for a number of human diseases including cardiovascular diseases (Anderson et al., 2000; Cavalca et al., 2001; Knekt et al., 2001), stroke (Yoo and Lee, 2001), peripheral arterial occlusive disease (Kang et al., 1992) and venous thrombosis (den Heijer et al., 1996). Elevated level of homocysteine (Hcy) plays also an important role in neural tube defects (Mills et al., 1996), the development of pregnancy complications (Nelen et al., 1997) and neurodegenerative diseases (Seshadri et al., 2002). Cardiovascular diseases are a major cause of mortality in developed countries. In recent years a number of studies were undertaken to understand homocysteine metabolism and mechanisms of its toxicity. Studies that provide insight into the metabolic pathways of homocysteine, regulation strategies and negative effects of elevated level of homocysteine, are crucial for the development of a new diagnostic and therapeutic methods.

Homocysteine is involved in conversions of methionine (Met) and cysteine (Cys) (Fig. 1). The immediate precursor of homocysteine is S-adenosylhomocysteine (SAH), which is hydrolyzed by SAH hydrolase (EC 3.3.1.1) to homocysteine and adenosine. In the next step homocysteine is remethylated to methionine by methionine synthase (EC 2.1.1.13) or betaine:homocysteine methyltransferase (EC 2.1.1.5). The first step of transmethylation reactions is the activation of methionine to S-adenosylmethionine (SAM) catalyzed by methionine adenosyltransferase (EC 2.5.1.6). Methyl group of SAM is subsequently transferred onto acceptor molecule and SAH is formed.

Homocysteine enters the transsulfuration pathway and is converted into cysteine by cystathionine β-synthase (EC 4.2.1.22) and cystathionine γ-lyase (EC 4.4.1.1). The transsulfuration pathway is present only in the liver, kidney, pancreas and small intestine (Brosnan et al., 2004).
Homocysteine can also enter the first step of protein biosynthesis. Because of structural similarity to methionine, homocysteine can be recognized and activated by methionyl-tRNA synthetase (MetRS). However, error-editing activity of MetRS does not allow homocysteine to be incorporated into protein. As a product of the editing reaction homocysteine thiolactone (HTL) is formed (Jakubowski and Fersht, 1981; Jakubowski, 2003, 2004). Subsequently, HTL may be hydrolyzed by thiolactonases to homocysteine (Jakubowski, 2000a; Perdziak et al., 2005; Zimny et al., 2005, 2006).

Homocysteine metabolism depends on the level of vitamins, which are required as cofactors by the enzymes involved in homocysteine turnover. Methionine synthase contains cobalamin (Vitamin B$_{12}$) as a prosthetic group and uses a folic acid derivative as a methyl group donor. Each of the transsulfuration pathway enzymes, cystathionine β-synthase and cystathionine γ-lyase, contain pyridoxal phosphate (Vitamin B$_6$) as a prosthetic group. Deficiencies of any of these vitamins are associated with hyperhomocysteinemia (Brosnan et al., 2004).

In human blood homocysteine exists in free or protein bound form. It may be either oxidized or reduced (Jacobsen, 1998) (Fig. 2). A major fraction of Hcy exists as protein N-linked homocysteine, with N-Hcy-hemoglobin and N-Hcy-albumin accounting for 75 and 22%, respectively, of the total N-Hcy-protein present in the human blood (Jakubowski, 2002b, 2005, 2006). Another homocysteine metabolite, HTL, represents up to 0.29% and up to 28% of plasma and urinary total homocysteine, respectively (Chwatko and Jakubowski, 2005a, b). A small fraction of homocysteine is also found as a free, reduced form. Most of oxidized form of homocysteine is bound to the protein, and the great bulk of this is linked to cysteine 34 (Cys$_{34}$) of albumin. The remainder occurs as disulfide, homocystine (Hcy-S-S-Hcy) and cysteinylhomocysteine (Cys-S-S-Hcy) (Jacobsen, 1998; Mudd et al., 2000). The term “total homocysteine” (“tHcy”) is commonly used to describe the pool...

![Fig. 1. Homocysteine metabolism. 1 – Methionine synthase, 2 – Betaine:homocysteine methyltransferase, 3 – Methionine adenosyltransferase, 4 – Methyltransferase, 5 – SAH hydrolase, 6 – Cystathionine β-synthase, 7 – Cystathionine γ-lyase, 8 – Thiolactonase, 9 – Met-tRNA synthetase, DMG – Dimethylglycine, THF – Tetrahydrofolate, 5-CH$_3$-THF – 5-Methyltetrahydrofolate](image-url)