Possible mechanisms of homocysteine toxicity

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Possible mechanisms of homocysteine toxicity. Hyperhomocysteinemia is a risk factor for cardiovascular disease in the general population. In chronic renal failure (CRF), plasma homocysteine levels rise when the glomerular filtration rate (GFR) is reduced 50%, and in uremia the majority of patients are hyperhomocysteinemic. The purpose of this study was to review possible mechanisms of homocysteine toxicity. Homocysteine, a sulfur amino acid found in blood in micromolar concentrations, can have toxic effects through a handful of general possible mechanisms. These mechanisms include oxidative stress (through the production of reactive oxygen species), binding to nitric oxide, production of homocysteinylated/acylated proteins, and accumulation of its precursor, S-adenosyl-homocysteine, a potent inhibitor of transmethylation reactions. Methyltransferase inhibition actually occurs in CRF and in uremia, and can have several functional consequences.

Hyperhomocysteinemia is a strong predictor of cardiovascular disease (CVD) in the general population [1]. A clear link between homocysteine and CVD was found in monogenic diseases called, as a whole, homocystinuria, in which plasma homocysteine concentration increases several-fold (400 to 500 μmol/L). More recently, mild (<16 μmol/L) hyperhomocysteinemia has been recognized as a CVD risk factor in the general population, and in a number of acquired conditions, such as chronic renal failure (CRF), where homocysteine levels can range from moderate (16 to 30 μmol/L) to intermediate (30 to 100 μmol/L). In a recent study, a population of 175 chronic hemodialysis patients followed for 29 months showed an increase in mortality rates, which was higher in the patients with the highest homocysteine levels at baseline [2]. Statistical adjustment for confounders and traditional and nontraditional risk factors was performed.

MECHANISMS OF ACTION

Hyperhomocysteinemia can be toxic for the endothelium; it enhances vascular smooth muscle cell proliferation, increases platelet aggregation, and acts on the coagulation cascade and fibrinolysis, directly inducing, or acting in a synergistic manner with other risk factors in creating a prothrombotic environment. It activates coagulation factor V, X, and XII, along with decreased activation of protein C and cell-surface thrombomodulin, and modulation of tissue plasminogen activator binding to its endothelial receptor, annexin II.

Proposed toxicity mechanisms are oxidation, nitrosylation, homocysteinylation, and hypomethylation. It has to be stressed that the different mechanisms of homocysteine toxicity are not mutually exclusive.

Oxidation

Thiols can auto-oxidize in the presence of transition metal catalysts and molecular oxygen, leading to the formation of reactive oxygen species (ROS). Homocysteine can induce a pro-oxidant action in vitro through hydrogen peroxide production during metal-catalyzed oxidation, and, in the presence of nitric oxide, the superoxide anion can form powerful oxidant peroxynitrite [3]. Formation of ROS could lead to lipid peroxidation, which initiates an inflammatory response and is involved in establishing foam cells, a key atherosclerotic lesion component. Studies performed in vitro have shown both a pro-oxidant and an antioxidant effect of homocysteine on LDL oxidation. Thiols can function as anti- or pro-oxidants, depending on the physical location (intra- or extracellular) of the oxidative damage occurring to lipoproteins. Depending on the experimental condition, homocysteine can both inhibit and promote LDL oxidation, and it has been speculated that its concentration (low = pro-oxidant, high = anti-oxidant) determines which activity will prevail [4]. However, phosphatidylcholine hydroperoxide, a product of lipid peroxidation, and P-selectin, a cell adhesion molecule and a marker of endothelial damage, did not change after a methionine load, suggesting that effects of homocysteine on endothelium are not mediated by oxidative stress mechanisms.

Key words: homocysteine, uremia, mechanisms of toxicity, hypomethylation.

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Homocysteine first displaces the cysteine residue present in position 34 of albumin. The favored reactions are as follows: the homocysteine sulfhydryl group reacts with the sulfur of the cysteine residue, forming the homocysteine-cysteine mixed disulfide and free albumin thiolate anion. Subsequently, albumin thiolate anion attacks the homocysteine-cysteine mixed disulfide on the homocysteine sulfur to form albumin-bound homocysteine and cysteine thiolate anion [14].

[5]. Clearly, more studies are necessary to elucidate the role of homocysteine on lipoprotein oxidation.

Homocysteine can impair endothelial cell function, which can be monitored by the reduction in endothelium-derived flow-mediated vasodilation by either a methionine load in healthy individuals, or in hyperhomocysteinemic patients [6–7]. Because of the protective effects exerted by administration of antioxidants such as vitamin C and E [8], and because of cell culture studies showing that homocysteine leads to considerable toxic effects mediated by ROS formation, it has been suggested that effects of homocysteine on endothelium occur via oxidative stress.

The activity of extracellular superoxide dismutase, an important antioxidant in vascular tissue, was measured along with homocysteine in homocystinuric patients and found to be positively associated with homocysteine levels. This strong relationship can be envisioned as a protective antioxidant response to homocysteine-induced oxidative damage [9].

Zappacosta et al [10] found that homocysteine produces only negligible quantities of hydrogen peroxide (1:4000 mmol of hydrogen peroxide/mole of homocysteine), and only when catalyst metal ions are present. Hydrogen peroxide was measured with a sensitive fluorometric method. According to these authors, homocysteine at micromolar concentrations does not act as a pro-oxidant but displays an antioxidant effect on cellular and chemical systems.

Importantly, only a small fraction of homocysteine (the oxidized form of homocysteine, also termed homocysteine homodimer) in blood is formed through a direct oxidative process, and albumin-bound homocysteine forms primarily through formation of an albumin thiolate anion (Fig. 1). The investigators conclude that the role of copper-catalyzed or ceruloplasmin-catalyzed autoxidation of homocysteine is probably a minor process in circulation [11–12]. It has been proposed that oxidative stress may generate within vascular cells, but not necessarily as a result of thiol oxidation [13]. In addition, perhaps folates, other B vitamins, and vitamin C and E are protective via mechanisms independent of their homocysteine-lowering or antioxidant effects.

The mechanism of homocysteine binding to plasma proteins is important since this amino acid is mostly transported though circulation in this form. Despite the rather high binding capacity of plasma proteins for thiols, a redistribution of protein-bound thiols takes place during hyperhomocysteinemia independently from any metabolic effect. An example of the displacement of protein-bound cysteine operated by homocysteine is given in Fig. 2.
concentration in this in vitro system. After dialfiltration to remove free non-protein-bound thiols in the sample not added with homocysteine, the peak of this amino acid almost disappears from dialfiltrate, confirming that most homocysteine is bound to proteins (compare Fig. 2 A and C). In the sample incubated with homocysteine, the proportion of free (non-protein-bound) homocysteine increases significantly (Fig. 2 B and D). Protein-bound homocysteine accounts for 99% and 68% of total, in the samples incubated without and with, homocysteine, respectively. Results shows that protein-bound cysteine and the dipeptide cysteine-glycine decrease in the sample incubated with homocysteine, compared to the sample in the absence of homocysteine (Fig. 2 B vs. 2 A). The percentage of protein-bound cysteine and cysteine-glycine is 49% (cysteine) and 44% (cysteine-glycine) in the high-homocysteine sample, compared to 86% (cysteine) and 81% (cysteine-glycine) in the low-homocysteine samples, respectively.

These results, obtained in a cell-free system, suggest that displacement may play a significant contribution, although in vivo effects due to metabolic adjustments need to be considered in a general mechanism of action. In favor of this interpretation is the reported existence of disulfide interchange in human serum albumin [11] (Fig. 1). Moreover, the situation described by our findings may cause an increased intracellular availability of free homocysteine.

Nitrosylation

Endothelial cells can detoxify homocysteine through release of nitric oxide, with an accompanying increase in Nos3 mRNA levels [3]. From the oxidation of homocysteine with nitric oxide, S-nitroso-homocysteine formation ensues. S-nitroso-homocysteine has vasodilatory and platelet antiaggregation properties, and does not support hydrogen peroxide generation. This represents a protective mechanism against the adverse effects of homocysteine. The scavenging effect of homocysteine is overcome in chronic exposure to high homocysteine, leading to a reduced production or availability of NO, leading to unopposed oxidative injury mediated by homocysteine and formation of peroxynitrite.

Homocysteines may affect glutathione peroxidase activity, thus altering the microenvironment in the propagation of ROS [14]. Endothelial glutathione peroxidase catalyzes the reduction of hydrogen and lipid peroxides to the corresponding alcohol, and it prevents the oxidative inactivation of NO. Homocysteine reduces mRNA levels of glutathione peroxidase, indicating that the expression of this enzyme protein is inhibited or downregulated.

Protein homocysteinlylation/acylation

A post-biosynthetic acylation of free amino groups in proteins is mediated by homocysteine thiolactone [15]. Plasma proteins, incubated in the presence of homocysteine thiolactone, form spontaneously homocysteinylated proteins, with complete disappearance of thiolactone from the medium after three hours. Consequences of protein homocysteinlylation are protein damage with an altered electrophoretic mobility, and loss of enzymatic activity (with protein denaturation) in several model systems, such as plasma proteins, methionyl tRNA synthetase, tripsyn, lysin oxidase, etc. Normally, it is not possible to measure homocysteine thiolactone in blood, probably because of its high reactivity and/or low concentration. However, when there is an increase in blood homocysteine, a parallel increase in homocysteinylated proteins can be observed. Protein homocysteinlylation could be one of the principal mediators of homocysteine toxicity, helping to determine structural and functional alterations at the molecular and cellular level [15].

Hypomethylation

An increase in plasma levels of homocysteine in uremic patients leads to a rise in the intracellular concentration of AdoHcy [16]. This thioether is the only homocysteine precursor and the natural inhibitor of all AdoMet-dependent transmethylation reactions. The ratio [AdoMet]/[AdoHcy] is a good indicator of the normal flow of methyl groups transferred from the methyl donor to methyl acceptors within the cell. The rise of AdoHcy concentration in erythrocytes of uremic patients, which is not paralleled by a rise of AdoMet concentration, gives way to a significant reduction of the [AdoMet]/[AdoHcy] ratio. This causes a significant impairment of AdoMet-dependent membrane protein carboxyl methylation reaction, catalyzed by protein carboxyl methyltransferase (PCMT, EC 2.1.1.77). This ubiquitous methylation reaction is involved in the repair of molecular damage, represented by L-isospartyl residues, spontaneously occurring in proteins through deamidation of labile asparagine residues. Several crucial transmethylation-dependent processes, in addition to protein repair in cells different from erythrocytes, can be affected by a reduction in the [AdoMet]/[AdoHcy] ratio. It has been shown that an increase in plasma homocysteine is associated with parallel increases in plasma AdoHcy and lymphocyte DNA hypomethylation, evaluated by HpaII digestion and cytosine-extension assay in normal women. Disruption of nonrandom DNA methylation pattern can lead to inappropriate gene expression and promotion of disease [17].

In uremic patients, plasma levels of damaged proteins, that is, proteins containing the L-isospartyl residues, are increased [18]. Folate treatment can lower, though not significantly, levels of damaged plasma proteins, meaning that other toxins besides homocysteine have a role in protein damage [18].

Metabolic repercussions of folate administration, 15 mg/day per os for two months, are to increase the eryth-
REFERENCES


CONCLUSIONS

Uremia can be considered a model of accelerated atherosclerosis, in which the high mortality rate is due to cardiovascular disease. These high rates cannot be entirely explained by the presence of traditional risk factors in these patients, but possibly by nontraditional risk factors, among them hyperhomocysteinemia. Four general mechanisms have been postulated to explain homocysteine toxicity, which can possibly integrate and are not mutually exclusive: oxidation, nitrosylation, acylation, and hypomethylation. However, no mechanism has been established, so far, which can provide an exhaustive biological basis for its toxic effects.

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