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# Homocysteine: New Aspects of an Ancient Enigma

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An extensive amount of literature has been written on homocysteine (Hcy), a sulfur-containing amino acid, related to methionine metabolism [1], either degraded via the remethylation pathway or converted, via the trans-sulfuration pathway, into cysteine. Nevertheless, once more, this biochemical compound is very well represented in a laboratory, and preclinically and pathologically, but the results deriving from its correction in clinical experience are poor [2].

## Hcy and Biochemical Functions

Hcy is related to the production of 5,10-methylenetetrahydrofolate, a fundamental step for the synthesis of thymidylate and purines and methionine, employing vitamin B<sub>12</sub> and folate as cofactors [3–6]. The S-adenosylmethionine (SAM) to S-adenosyl-L-homocysteine (SAH) ratio defines the methylation potential of a cell [7]. If Hcy is allowed to accumulate in normal conditions, it will be rapidly metabolized to SAH [8, 9]. Whenever there is a methionine deficit, Hcy can be re-methylated to form methionine, by the employment of N<sup>5</sup>,N<sup>10</sup>-methylenetetrahydrofolate [10]. If there is an adequate amount of methionine, Hcy is employed for the production of cysteine, mediated by cystathionine-beta synthase, with pyridoxine as a cofactor [10]. Therefore, the accumulation of Hcy is dangerous when it occurs in the absence of folate as a cofactor. Recent studies are generally confident with the fact that lifestyle conditions (such as smoking, alcohol

consumption, physical inactivity) may help the elevation of Hcy [11–15]. Genetic condition of hyperhomocysteinemia (HHcy) has been considered a significant risk and often fatal factor [5, 16]. Undoubtedly, the methylation reactions are strongly necessary for the brain, given that SAM is the sole donor in numerous methylation reactions, involving proteins, phospholipids, and biogenic amines [16, 17], and for packaging of many phospholipids [18]. This way, alterations of methylation with consequent Hcy accumulation lead to many congenital neural tube and central nervous system alterations [19]. The causative factors of accumulation of Hcy in healthy adult life can be diverse, due to various genetic defects or to the defects of vitamin B<sub>12</sub> and folate [20]. A physiological increase of Hcy occurs in the brain (and CSF) and the plasma, within the aging process, and more evidently inside several neurological diseases [21].

## Hcy and Clinical Perspectives: Neurodegeneration

It has been proven that Hcy could be linked to neurodegeneration; Hcy (in tight relationship with higher glycine levels in the brain) is an agonist of the endogenous glutamate receptors, NMDA receptors [22], influencing calcium influx [16, 23, 24], as well as through a direct activation of the group I metabotropic glutamate receptors [25]. Much evidence suggests that Hcy potentiates the toxicity of Aβ<sub>42</sub> deposition [26–28] and increases amyloid's toxicity on the smooth vascular cells in the brain

[29]. Moreover, HHcy upregulates presenilin 1, which promotes APP synthesis [30, 31]. Finally, Hcy is related to the phosphorylation process of tau. The protein phosphatase methyltransferase 1, whose methylation is SAM-dependent, regulates the activity of the protein phosphatase methyltransferase 2A, which acts as a dephosphorylating system for tau protein [32–34]. Hence, the reduced methylation capacity increases the hyperphosphorylation of tau protein, determining microtubule disaggregation, their precipitation, and the deposition of the neurofibrillary tangles.

### **Hcy and Clinical Perspectives: Inflammation and Oxidative Process**

The most fascinating, irrefutable aspect of Hcy is its pro-inflammatory and pro-oxidative role. Being that the SAM-to-SAH ratio is the expression of the methylation potential of a cell, “HHcy tends to decrease the methylation potential” [10]. Therefore, Hcy can induce a global DNA hypomethylation and suppress the transcription of cyclin A in endothelial cells; at the same time, Hcy leads to upregulation of some other genes, causing an increase in p66shc expression in endothelial cells, inducing oxidant stress [7, 10]. It is widely accepted that Hcy leads to an induction of m-RNA and protein expression of C-reactive protein (CRP), augmenting the NR1 subunit of NMDA receptor expression; therefore, Hcy can promote a pro-inflammatory response in vascular smooth muscle cells of small brain arteries by stimulating CRP production, usually enhanced by a combined NMDA-ROS-erk1/2/p38-nfKBeta signal pathway [35]. Recently, a well-conducted study [36] demonstrated that cultured cell incubation with Hcy determined cell death at 80  $\mu$ M Hcy exposure after 5 days; quite impressively, cell exposure to Hcy at lower concentrations for 5 days raised to a 4.4-fold increase in reactive oxygen species (ROS) production. Hcy leads to a general upregulation of p21 and p-16 after 5 days of Hcy incubation, inducing a reduction of 35% of pRB, checkpoint regulators of G1 cell-cycle phase. In response to the HHcy level, endothelial cells produce nitric oxide (NO) to induce the formation of S-nitroso-Hcy, which acts as a protector of endothelium; however, the chronic exposure to Hcy induces a final diminishment of NO [37]. Therefore, endothelial dysfunction due to HHcy results from a disruption in the cellular integrity, leading to impaired endothelium-dependent relaxation, mainly due to a reduction in the NO bioavailability [37, 38], to a stimulation of muscle cells, and to the

promotion of inflammatory response, testified by an increase in C-reactive protein and cysteinyl leukotrienes, associated to an increment of HMG-CoA reductase activity [38]. The activities of methionine synthase that mediate the clearance of Hcy are linked to the redox potential of the cells [39]; usually, more Hcy is converted into cysteine and glutathione. A disruption of the cystathionine-beta synthase causes altered redox homeostasis and alteration of oxidative repairing process [40]. The disruption of the redox system in vascular and neuronal cells [41] accelerates the lipid peroxidation sequel of events [41–45].

Interestingly, multiple traumatism and secondary septic status associated with a systemic inflammatory response have been associated with HHcy, and the constancy of this report is related to a poor clinical outcome [46]. Curiously, this condition is not determined by a loss of folate and B<sub>12</sub>. Therefore, it can be argued that the pro-inflammatory condition of these patients leads to strong activation of macrophage system cascade by Hcy, with a consequent release of ample amounts of ROS, potentiating the oxidative stress [46]. HHcy activates B lymphocytes; this process seems to determine an increase of pyruvate kinase muscle isozyme 2 (PKM-2) in B cells. PKM-2 seems to suggest the so-called metabolic accelerated initiation of atherosclerosis cascade mediated by HHcy, in vivo and in vitro [47–49]. Cultured macrophage cells exposed to Hcy showed a memory response, probably induced by epigenetic mutations [48], which influences the expression of promoter genes regulating inflammatory response and endothelium atherogenesis. A single study demonstrated an in vitro Hcy-dependent alteration of the transcriptional repression of fibroblast growth factor 2 [50]. As written above, Hcy acts on NMDA receptors: they are not only found in neurons but also on neutrophils and macrophages. The activation of these peripheral receptors, as well as in the cerebral context, rises the cytoplasmatic calcium influx and activates a pro-inflammatory cascade, with an accumulation of ROS species [51, 52], which induces an upregulation of the nuclear factor kappa B, considered as one of “the master regulators of the expression of inflammatory genes” [52].

### **Hcy and Clinical Experiences**

An increasing amount of evidence showed that Hcy is associated with different kinds of cardiovascular and cerebrovascular diseases [53, 54]. It has been reported that HHcy relates with stroke, promoting cerebrovascu-

lar atherosclerosis and atherothrombosis via upregulation of matrix metalloproteinases-9 (MMP-9) expression, which takes responsibility for atherosclerotic plaque instability and even their ruptures [55, 56]. Clinical trials and studies failed to demonstrate univocal and conclusive results, either preventing HHcy through the supplementation of vitamin B<sub>12</sub>, folate or both in patients or a healthy population, or considering HHcy as a real target of prevention. Much criticism may be led towards the trials implemented [15].

## Take-Home Messages

In a debate which lasts many decades, at the moment, the only mandatory take-home messages are:

- 1 Hcy cannot be considered as a definite marker of vascular risk factor or neurodegeneration.

- 2 Hcy can be employed as a target to prevent the increase of endothelial damage and of vascular comorbidities.
- 3 Hcy is an inflammation marker, which seems to define adverse or poor outcomes in many clinical scenarios (sepsis, stroke, hemorrhage).
- 4 More studies should be done, more data should be collected, and more defined prospective population studies should be implemented, in order to finally have the solution of this enigma [57].

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