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Hyper-homocysteinemia: a novel risk factor or a powerful marker for cardiovascular diseases? Pathogenetic and therapeutical uncertainties

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Abstract Increased homocysteine levels can be responsible for arterial ischemic events, such as MI, stroke or peripheral vascular disease. Homocysteine is metabolized by two pathways: re-methylation and trans-sulfuration. Both involve folic acid, and vitamins $B_{6-12.}$ Several studies assumed that the folates and vitamins B supplementation or dietary source to normalize plasma homocysteine. But, even if tends to normalize homocysteine levels, lowering homocysteine by B-group vitamins and/or folates does not reduce cardiovascular risk. In fact, recent reports confirmed that hyper-homocysteinemia is not directly responsible for cardiovascular disease, but is merely present in individuals suffering for acute and/or chronic cardiovascular events, as a collateral finding. Reduced methylation potential (MP) [due to decreased S-adenosyl-methionine (AdoMet)/S-adenosyl-homocysteine (AdoHcy) ratio] induced by the elevated plasma homocysteine levels seems to be the true responsible for cardiovascular diseases (CVD). The pathogenic mechanisms responsible for CVD appear to be dependent of DNA hypomethylation inducing an inhibition of cyclin A transcription and a reduction of endothelial cells growth. But, other human studies performed in a wide range are requested.

Keywords Homocysteine · Cardiovascular risk · Folic acid/vitamins B_{6-12} hypo-methylation

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Introduction

According to the American Heart Association (AHA), normal homocysteine concentrations are included between 5 and 15 μmol/l, intermediately homocysteine serum levels are between 31 and 100 μmol/l, and severely elevated concentrations are >100 μmol/l. But, an area of lightly positive values is included among 15 and 31 μmol/l. In fact, circulating homocysteine serum levels >15 μmol/l but <30 μmol/l also increase the risk of developing atherothrombotic cerebro-vascular, coronary and peripheral vascular diseases, especially if the homocysteinemia-genic mutation is present in homozygotic fashion, for the coexistence of another coagulative genetic mutation (frequently factor V Leiden), and/or for contemporary cigarette smoking [1]. Hyperhomocysteinemia has been also identified as a mediator of other clinical manifestations different from vasculopaties, such as dementia-type disorders [2], Alzheimer disease [3], premature osteoporosis-induced bone fractures [4], mental retardation, and premature diabetic retinopathy [5]. Finally, hyperhomocysteinemia can be responsible for neural tube defects in pregnant women [6]. In this report, the relationship between high homocysteine levels and ischemic arterial events is illustrated. Its serum reduction by B-vitamins and/or folates without contemporary vascular-risk reduction and the most recent pathogenetic mechanisms by which hyperhomocysteinemia induces early atherosclerosis are also reported.

Homocysteine is a sulfur-containing nonproteinogenic aminoacid biosynthesized from Methionine. In its metabolic cycle, serum levels of homocysteine undergo re-methylation to methionine in a reaction catalyzed by the vitamin B_{12}-dependent methionine synthase. This reaction is catalyzed by the enzyme MethyleneTetraHydroFolate Reductase (MTHFR). But, homocysteine can be also

trans-sulfurated to cysthathionine and subsequently to cysteine. Trans-sulfuration requires the vitamin B_6 dependent enzyme cystathionine β-synthase. These two metabolic pathways of homocysteine are reported in the following schema (Fig. 1). Increasing values of homocysteine can be obtained for a reduced MTHFR (transmethylation) enzymatic activity (that is the most common form of genetic hyperhomocysteinemia). On the contrary, hyperhomocysteinemia consequent to reduced trans-sulfuration is rather rare. Thus, deficiencies both of vitamins B_6, B_{12} and folic acid are associated with an elevation of homocysteine plasmatic levels [7]. Other disorders characterized by increased homocysteine serum concentrations include renal disease, hypothyroidism, cancer, psoriasis, and certain drugs (contraceptive drugs, methotrexate, carbamazepine, sulphosalazine, etc.). Some factors as cigarette smoking, coffee abuse, and arterial hypertension may contribute to speed and worse the negative effects of hyperhomocysteinemia. With regard to cigarette-smoking, a study by O'Callaghan and colleagues provided evidences for an amplifying effects of smoking on homocysteine-associated cardiovascular risk. Smokers with plasma homocysteine above 12 µmol/l had a 12-fold increase of cardiovascular risk respect to non-smokers with plasma homocysteine less than 12 µmol/l [8]. Nevertheless, the whole mechanisms mediating the synergy between homocysteine vascular-damages and smoking remain unclear too.

Mechanisms of thrombophilic effects

With regard to the vascular damages, homocysteine is able to induce numerous effects: it reduces nitric oxide bioavailability by stimulating the formation of reactive oxygen species (ROS) [9]. Hyper-homocysteinemia causes a reduction of vessel radius by thickening the arterial wall [10]. In addition, it increases matrix metalloproteinase activity resulting in an alteration of the elastin/collagen ratio and reduced compliance of the arterial walls [11]. It is known that homocysteine also increases the platelets' adhesion and aggregation, favouring the formation of the arterial thrombi [12, 13], (Fig. 2). Finally, homocysteine acts on some coagulative factors, as antithrombin III, protein C reactive (PCR), factor VII action, and PTA, favouring the coagulative effects. It also increases LDL oxidization [14]. Another potential mechanism is the stimulatory effect on smooth-muscle proliferation [13]. Because of these actions, hyperomocysteinemia may be associated with both arterial and venous thrombosis, even if the arterial (or white) thrombi happen more frequently than the venous (or red) thrombi. It is known that arterial thrombi are primarily constituted by platelets, little amount of fibrin, and develop at sites of vessel wall injury in presence of high-velocity blood flow. On the contrary, venous thrombi are primarily composed of fibrin and trapped erythrocytes. They include few platelets, and grow in areas of haematic stasis. For these remarks, hyperhomocysteinemia preferably induce arterial ischemia, with consequent antiplatelets'-prevention therapy. With respect to the incidence of negative vascular effects, although severe hyperhomocysteinemia in the general population rarely was found, mild hyperomocysteinemia was estimated to be 5% and among individuals with symptomatic CAD, previous ischemic stroke or peripheral vascular disease, it increased up to 13–47% [15].

Cardiovascular disorders

In 1969, Mc Cully demonstrated that premature atherosclerosis is often associated with severe hyperhomocysteinemia. This condition frequently leads to premature coronary, cerebral or peripheral vascular impairments [16–19]. For that, this author firstly proposed the "homocysteine hypothesis of atherosclerosis". Successively, den Heijer and colleagues evidenced that mild hyperhomcysteinemia is an independent risk factor for venous

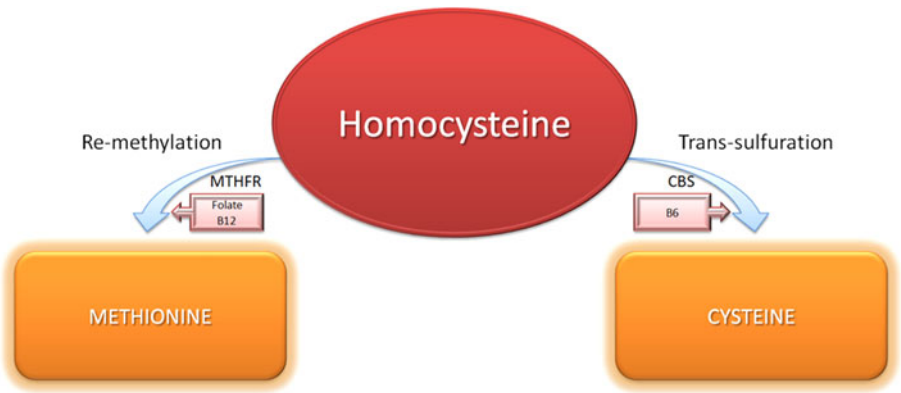

Fig. 1 Metabolic reduction of homocysteine plasma concentration—The amino acid may be re-methylate to Methionine or trans-sulfurate to Cysteine. Re-methylation requires the enzyme MehylenelTetraHydroFolate Reductase (MTHFR), that has as co-factor folic acid and vitamin B_{12}. Trans-sulfuration happens by Cystathionine β-Synthase (CBS) catalizyed by vitamin-B_6

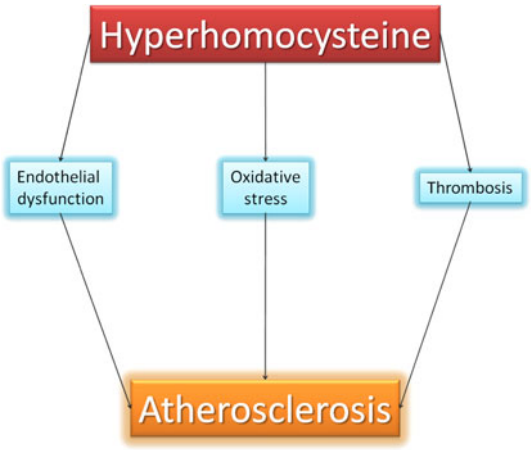

Fig. 2 Classic mechanisms leading to the atherosclerosis from a condition of hyperhomocisteine

thromboembolism [14] even if conflicting data exist whether the risk of venous thromboembolism markedly increased in patients with hyper-homocysteinemia alone. The combination of hyperhomocysteinemia and factor V Leiden (that more frequently occurs) further increases the relative risk of venous thromboembolism. Recently, Hassan et al. demonstrated that hyperhomocysteinemia is an independent risk factor for cerebral small vessel disease, acting via endothelial dysfunction. It causes focal lacunar infarction and more diffuse ischaemia, referred to as leukoaraiosis [20]. Jeong and colleagues evidenced a direct influence of elevated homocysteine even on the large arteries, such as internal carotid artery [21].

Therapy of hyperhomocysteinemia

Numerous randomized trials investigated the effect of folic acid and/or vitamins B_6 and B_{12} supplementation on multiple markers of CVD. A Homocysteine Studies' Collaboration meta-analysis indicated risk reduction for vascular disease in patients with hyperhomocysteinemia treated with folic acid, vitamins B_6 and B_{12}. Specifically, treatment with folic acid and/or B vitamins reduced the risk of ischemic heart disease by 11% and for stroke by 19% per 3 μmol/l reduction in homocysteine concentration [22]. The reduction of hyperhomocysteinemia was also obtained in patients with renal insufficiency and increased levels of homocysteine. It is known that renal failure is strongly associated with end-stage renal disease (ESRD), because renal insufficiency contributes to homocysteine retention, even if the complete mechanism of renal failure in inducing hyper-homocysteinemia is unresolved [23]. In this connection, in a report, approximately 85% of hemodialysis patients evidenced homocysteine levels above the 95th percentile for normal controls. Supplementation with doses of folic acid greater than 15 mg/day may possibility reverse these values [24]. To evaluate the efficacy of supplementation with high dose folic acid and with vitamin B_{12} in lowering plasma total homocysteine concentrations in hemodialysis patients, a recent study demonstrated that the giving of 15 mg/day of folic acid +1 mg/day of vitamin B_{12} resulted in a 30% reduction in plasma homocysteine concentrations [25]. This finding is also supported by the results by Koyama et al. [26] and further confirmed by Manns and coworkers [27]. But, these same results were not found by Trimarchi [28].

With reference to the homocysteine-lowering and coronary restenosis, a first study performed in patients with baseline normal-to mild hyperhomocysteinemia provided the evidence of a reduction of angiographic restenosis 6 months after coronary angioplasty and stenting associated with vitamin supplementation [29]. But, the Swiss Heart Study demonstrated that homocysteine-lowering therapy did show significant risk reduction in major adverse cardiovascular events in patients treated with angioplasty, with or without stenting [30]. Finally, Lange et al. evidenced that the multivitamin therapy, although reduced serum homocysteine levels, was associated with a paradoxical increase of restenosis and major adverse cardiac events at 6 months, particularly in patients with homocysteine levels in the normal range, whereas slight benefits were observed in patients with elevated homocysteine [31]. In the 14-year follow-up, the Nurses' Health Study Researchers suggested that intake of folic acid and vitamin B_6 may have positive impact on the primary prevention of heart disease among women and the maximum benefit would be achieved at folic acid intake of at least 400 μg/day [32]. More recently, Investigators of the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) concluded that folic acid/vitamin B12 supplementation did not produce a benefit for major vascular events overall or any of the subgroups, nor benefit shown for incidence of stroke prevention [33]. Contrarily to all these results, one study evidenced that homocysteine levels typically rise after an acute vascular event in response to tissue damage or repair and remain elevated for months. These findings suggest that elevated homocysteine may be a consequence rather than a cause of vascular disease [34]. Except for the last report, the results obtained for all cited studies seems to demonstrate the importance of hyper-homocysteinemia as novel risk factor for cardiovascular diseases. But, in a recent report Kaul et al. [35] did not confirm this hypothesis. These affirm that it is unclear whether a causal relationship exists between homocysteine and cardiovascular risk, if homocysteine is related to other confounding cardiovascular risk factors or is marker of existing disease burden. For these reasons, the AAs. also conclude that

routine screening for elevated homocysteine is not yet recommended. On the contrary, de Ruijter et al. demonstrated that high levels of homocysteine predict risk of death and morbility for cardiovascular disease in older people better than any conventional measure of risk, including cholesterol, blood pressure or smoking [36].

With reference to the conflicting results on cardiovascular risk obtained with the acid folic and vitamin B_{12} supplementation some trials, such as The Vitamin Intervention For Stroke Prevention (VISP) [37], Heart Outcomes Prevention Evaluation (HOPE)-2 [38], and Norwegian Vitamin Trial (NORVIT) [39] have demonstrated that folic acid and vitamin B supplementation decreased homocysteine levels, without significant reduction in primary endpoint outcome measure as MI, stroke and peripheral disease. Concerning that, in a recent meta-analysis, Clarke and coworkers re-affirmed that lowering homocysteine levels by an average of 25% for an average of 5 years has no significant effects on the incidence of major vascular events. For disagreement between the reduction of homocysteine levels and the persistence in these same of cardiovascular risk after folic acid and $B_{12\text{-}6}$ vitamins supplementation, hyper-homocysteinemia seems to be a marker rather than a factor directly responsible for increased cardiovascular risk. Thus, its specific treatment with folates and vitamin $B_{12\text{–}6}$ appears to be not necessary for reduce the cardiovascular risk [40].

Contemporary constructs

In support of the hypothesis that hyperhomocysteinemia is a biomarker rather than a real risk factor of cardiovascular disease, a recent report by Kim et al. affirms that hyperhomocysteinemia may be responsible for a reduced re-methylation capacity which would cause decreased genomic DNA methylation [41]. Therefore, hyperhomocysteinemia appears not to be a direct cause of increased and premature atherosclerosis. This is a consequence of reduced DNA methylation that could play a causative role in atherogenesis [42]. In the homocysteine metabolic pathway, dietary methionine is converted to the methyl donor S-adenosylmethionine (AdoMet) and is demethylated to S-adenosylhomocysteine (AdoHcy) and homocysteine (Fig. 3). The ratio of AdoMet to AdoHcy, also defined as the Methylation Potential (MP), indicates the flow of methyl groups within the cells. As recently shown, chronic elevations of plasma total homocysteine correlate with increased AdoHcy levels and decreased MP in plasma. In fact, the increase in AdoHcy causes a feedback inhibition of AdoMet-dependent methyltransferases, including the DNA methyltrasferases [43, 44]. The low MP, in turn, is associated with a decreased DNA methylation [45]. In the transsulfuration pathway, homocysteine is converted to cystathionine by the enzyme cystathionine β-synthase (CBS). The lack of CBS activity in endothelial cells may also enhance their sensitivity to AdoHcy accumulation and, consequently, to DNA hypomethylation. This state may alter methylation-dependent gene regulation and may have unwanted effects on a some organ systems. These findings are in line with the hypothesis that the pathogenic role of hyperhomocysteinemia in vascular disease might be caused by AdoHcy accumulation and DNA hypomethylation [46]. Therefore, homocysteinemia is assumed as an independent marker for increased cardiovascular risk and DNA hypomethylation may be one of the main responsible mechanisms. However, the biochemical bases by which DNA hypomethylation contributes to CVD remained largely unknown for a long time. Recently, Jamaluddin et al. [47] demonstrated that hyperhomocysteinemia exerts highly selective inhibitory effects on cyclin A transcription and endothelial cells growth through a hypomethylation related mechanism which blocks cell cycle progression and endothelium regeneration. That happens by DNA methyltransferase 1 activity reduction. It is known that cyclin A levels are increased in a variety of tumors, in normal myocites growth, in hypertrophy and in atherosclerosis. Its suppression appears as a mechanism responsible for growth-inhibition in endothelial cells contributing to cardiovascular diseases (CVD).

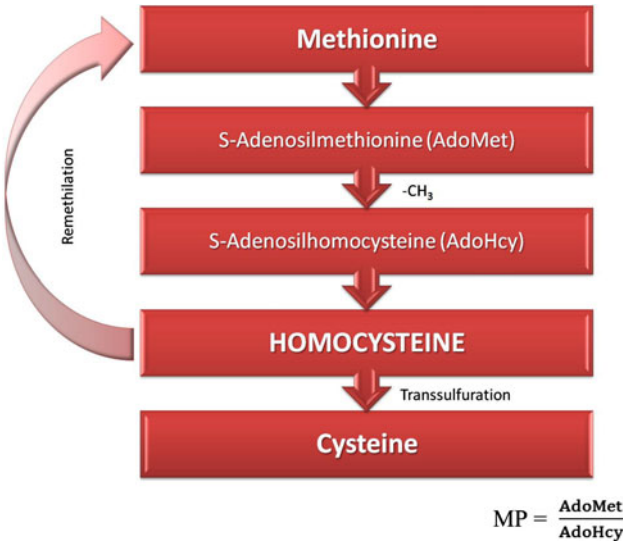

Fig. 3 Homocysteine metabolic pathway-Increased of homocysteine plasma levels induce (for a feed-back mechanism) a reduction of methyl-groups release. The lack of trans-sulfuration reaction also reduces-CH_3 release. *MP* methylation potential

It is known that the cell cycle consists of four phases: G_0 (quiescent or resting phase), G_1 phase (in which cells increase in size), S phase (also called synthesis phase), G_2 phase (known as interphase); M phase (mitosis). Two classes of regulatory molecules: cyclins and

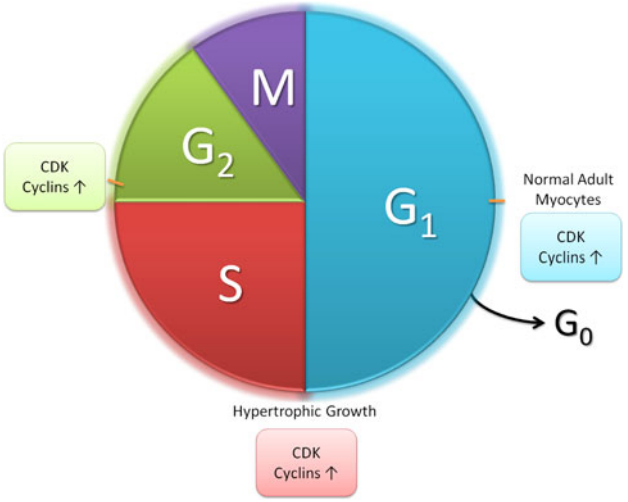

Fig. 4 Schema of the cell cycle. *M* mitosis; G_1 Cells increase in size; G_0 Resting phase where the cell has stopped dividing; *S (synthesis)* DNA replication occurs during this phase.; G_2 during the gap between DNA synthesis and mitosis, the cell continue to grow. Cyclins and cyclin-dependent kinases (CDKs) determine a cell's progress through the cell cycle

cyclin-dependent kinase (CDK) determine a cell's progress through the cell cycle (Fig. 4). Cyclins have no catalytic activity and CDKs are inactive in the absence of a partner cyclin. As regard the relationship among homocysteine and atherosclerosis. It was suggested that endothelial injury may is induced by hyperhomocysteinemia. Homocysteine inhibits endothelial cells' growth and promotes vascular smooth muscle cell (VSMC). Previously, it was found that the growth-inhibitory effect of homocysteine in endothelial cells is explained by reduced cyclin A transcription and associated methylation inhibition [48]. Reduced endothelial-cell growth, reduced re-endothelialization and VSMC proliferation in turn favour early atherosclerotic process, as represented in Fig. 5.

Conclusive remarks

These acquisitions about vascular derangements in hyperhomocysteinemia indicate that this is not only a cause responsible for early atherosclerosis, but also a powerful biomarker for the degenerative disease. In addition, the increased homocysteine serum levels not only indicate a simple thrombophilia, but are also able to determine premature atherosclerosis. Finally, acid folic and/or B_{6-12} vitamins therapy only induce a lowering of homocysteine serum concentrations, but is unable to reduce the incidence of atherosclerotic processes and their acute manifestations. But, further observations performed in humans are requested to clarify the underlying mechanisms and to provide suggestions for future therapeutic strategies.

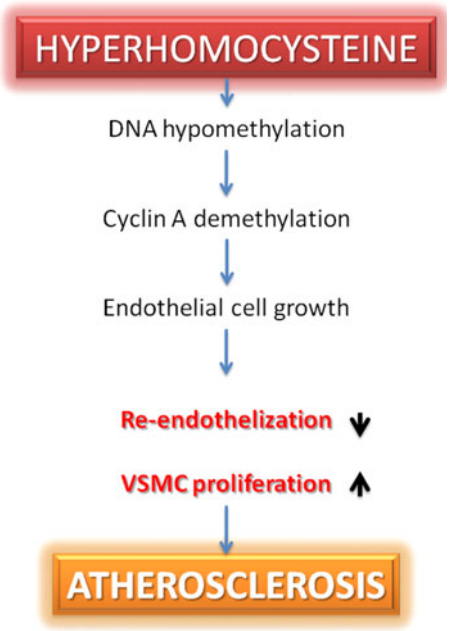

Fig. 5 Schematic representation of the contemporary mechanisms of hyperhomocysteine favouring DNA hypomethylation by the cyclins demethylation. That favours endothelial cells' growth and vascular smooth muscle cell (VSMC) proliferation, until the atherosclerosis

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