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INVITED REVIEW

Hyperhomocysteinemia impairs regional blood flow: involvements of endothelial and neuronal nitric oxide

Noboru Toda[1] · **Tomio Okamura**[2]

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Abstract Increasing evidence support the idea that hyperhomocysteinemia (HHcy) is responsible for pathogenesis underlying cerebral, coronary, renal, and other vascular circulatory disorders and for hypertension. Impaired synthesis of nitric oxide (NO) in the endothelium or increased production of asymmetric dimethylarginine and activated oxygen species are involved in the impairment of vasodilator effects of NO. Impaired circulation in the brain derived from reduced synthesis and actions of NO would be an important triggering factor to dementia and Alzheimer's disease. Reduced actions of NO and brain hypoperfusion trigger increased production of amyloid-β that inhibits endothelial function, thus establishing a vicious cycle for impairing brain circulation. HHcy is involved in the genesis of anginal attack and coronary myocardial infarction. HHcy is also involved in renal circulatory diseases. The homocysteine (Hcy)-induced circulatory failure is promoted by methionine and is prevented by increased folic acid and vitamin B_6/B_{12}. Eliminating poor life styles, such as smoking and being sedentary; keeping favorable dietary habits; and early treatment maintaining constitutive NOS functions healthy, reducing oxidative stresses would be beneficial in protecting HHcy-induced circulatory failures.

Keywords Hyperhomocysteinemia · Nitric oxide · Cerebral blood flow · Coronary blood flow · Oxidative stress

✉ Noboru Toda
n.toda.toyama-bldg@orion.ocn.ne.jp

[1] Toyama Institute for Cardiovascular Pharmacology Research, 7-13, 1-Chome, Azuchimachi, Chuo-ku, Osaka 541-0052, Japan

[2] Department of Pharmacology, Shiga University of Medical Science, Seta, Otsu, Shiga 520-2192, Japan

Abbreviations

ACh	Acetylcholine
Aβ	Amyloid-β
AD	Alzheimer's disease
ADMA	Asymmetric dimethylarginine
AβPP	Aβ-protein precursor (AβPP)
DDAH	Dimethylaminohydrolase
EPC	Endothelial progenitor cell
GABA	γ-Amino butylic acid
Hcy	Homocysteine
HHcy	Hyperhomocysteinemia
NO	Nitric oxide
PPARγ	Peroxisome proliferator-activated receptor-γ
SOD	Superoxide dismutase

Introduction

Homocysteine (Hcy) is a thiol-containing amino acid that is formed from methionine, an essential amino acid contained in large quantities in meat. Recent evidence indicates that elevated plasma level of Hcy is a risk factor for occlusive artery disease, especially in the brain [9, 17], the heart [16, 55, 69], and the kidney [48].

Cerebral blood flow and peripheral circulation are controlled by vasodilator factors, including endothelium-derived nitric oxide (NO), prostacyclin, endothelium-derived hyperpolarizing factors, and vasodilator peptides as well as vasoconstrictors, such as vasoconstrictor prostaglandins, oxidative stress, and asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS). Neural control of cerebral blood flow is regulated mainly by parasympathetic nitrergic nerves, whereas adrenergic vasoconstrictor nerves are mainly involved in peripheral blood flow control; coronary blood flow is controlled by norepinephrine liberated from

adrenergic nerves that induces vasodilatation via activation of adrenergic β-adrenoceptors and vasoconstriction by α-adrenoceptor stimulation [77]. Hyperhomocysteinemia (HHcy) interferes with cerebral and peripheral blood flow mainly by impairment of actions and syntheses of vasodilator factors, such as NO [15, 26, 45, 48]. HHcy also increases oxidative stress and accumulate ADMA, which acts as an endogenous inhibitor of NOS [72, 78], and prevents expression of endothelial dimetylaminohydrolase (DDAH), the main catabolic enzyme of ADMA [23, 49, 70]. Dietary folic acid decreases the plasma HHcy levels [44]. Folate supplementation to adults was found to protect against ischemic stroke or myocardial infarction [37].

Here, we will review and discuss the importance of current research on the modulation of regional blood flow or vascular tone, mainly in the cerebral, coronary, and renal vasculatures, maintained by basal and stimulated release of NO from the endothelium and nitrergic nerves or by oxygen radicals following HHcy in humans and experimental animals.

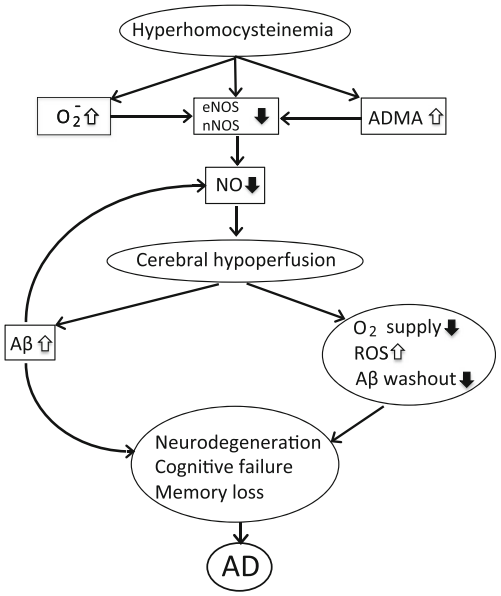

Fig. 1 Possible roles hyperhomocysteinemia in inhibiting eNOS and nNOS functions, impairing cerebral blood flow, and increasing Aβ deposition in the pathogenesis of Alzheimer's (AD) disease. O_2^- superoxide anion, *ADMA* asymmetric dimethylarginine

General features of vascular function in hyperhomocysteinemia

Hcy is an independent risk factor for atherothrombosis [50]. Hcy is a nonprotein amino acid generated during nucleic acid methylation and demethylation of methionine (Fig. 1). Folic acid decreases Hcy levels by remethylating the Hcy to methionine. HHcy is a defined state connected to the increased risk of atherothrombotic, atherosclerotic, and vascular constrictive effects, largely as a result of facilitating the generation of hydrogen peroxide from oxygen. Endothelium-derived relaxing factor (EDRF) [31] or NO is suggested to S-nitrosate Hcy, rendering it nontoxic to the endothelium [67]. Prolonged exposure of endothelial cells to Hcy impaired vasodilator responses to EDRF, whereas S-NO-Hcy neither supported H_2O_2 generation nor impaired the response to EDRF [68]. These authors suggested that the normal endothelium modulates the adverse effects of Hcy by releasing EDRF. EDRF/NO acts as a detoxifier of Hcy, both by limiting the generation of hydrogen peroxide and by serving as a reducing equivalent for the nitrosation reaction [83]. Responses of resistance vessels to endothelium-dependent vasodilators, acetylcholine (ACh) and ADP, were impaired in cynomolgus monkeys with diet-induced HHcy [45]. These authors suggested that altered vascular function may contribute to vasospasm and thrombosis. Increase in vascular oxidant stress through imbalanced thiol redox status and inhibition of antioxidant enzymes by Hcy results in decreased bioavailability of endothelial NO via oxidative inactivation [85, 87]. Vasodilator effects of endothelium-derived NO and exogenously applied NO donors are compromised by HHcy in anesthetized rats [30]. Elevated plasma levels of ADMA and symmetric dimethylarginine are associated with an increased risk of cardiovascular events [8]; the concentrations of these endogenous NOS inhibitors in rat plasma are actually decreased upon methionine administration.

Endothelium-dependent flow-mediated dilation in brachial arteries was significantly lower in HHcy adult subjects than in those with low Hcy levels, suggesting that HHcy is an independent risk factor for arterial endothelial dysfunction [89]. In the forearm circulation of healthy subjects, increased Hcy plasma levels reduce NO availability by producing oxidative stress; in essential hypertensive patients, the presence of hypercholesterolemia causes a further reduction in endothelium-dependent vasodilatation by exacerbating oxidative stress [73]. Atherogenic effects of Hcy appear to depress endothelial function through NO-dependent mechanisms in humans [34].

Oral methionine loading increased Hcy levels and resulted in a decrease in flow-mediated vasodilatation of the brachial artery in healthy subjects, suggesting that acute HHcy impairs endothelial function [1]. Oral methionine raises plasma Hcy and impairs flow-mediated endothelium-dependent brachial artery vasodilatation in healthy adults [6]. In healthy volunteers, methionine increased plasma Hcy and reduced flow-mediated, NO-mediated brachial artery dilatation [14]. Folic acid and vitamin B_{12} supplementation improved endothelial function in patients with coronary heart disease [16]. In hyperhomocysteinemic patients, folic acid treatment for 12 months lowered elevated plasma Hcy and reversed Hcy-induced impairment of NO vasoreactivity. Acute HHcy decreases flow-mediated vasodilatation of the brachial artery in

healthy subjects; pretreatment with aged garlic extracts diminished the adverse effects of acute HHcy [86].

Cerebral circulation

In anesthetized rats, cerebrocortical blood flow was reduced by superfusion with Hcy-Cu^{2+} but not with Cu^{2+} alone, and the effect of Hcy-Cu^{2+} was prevented by co-administration of superoxide dismutase (SOD); Hcy-Cu^{2+} attenuated the vasodilator response to NO-dependent vasodilators, such as ACh and hypercapnia, suggesting that O_2^- generated by the reaction of Hcy with Cu^{2+} inhibits NO-related cerebrovascular responses by scavenging NO [90]. Lee et al. [43] provided evidence suggesting that endothelial dysfunction induced by HHcy is mediated through impairments of endothelial NOS (eNOS) activity in cerebrovascular endothelial cells and that these effects are ameliorated by dietary folic acid supplementation in rats. Dilatation to ACh of cerebral arterioles in mice fed a high-methionine diet was impaired, as compared with those fed a control diet; the vasodilator responses were restored toward normal by the superoxide scavenger tiron, suggesting that superoxide is a key mediator of endothelial dysfunction in the cerebral circulation during HHcy [22]. Betzen et al. [7] suggested that oxidative stress upregulates N-methyl-D-aspartate receptor on murine cerebrovascular endothelium and heightens susceptibility to glutamate-induced blood–brain barrier disruption. Hcy-mediated brain "microvascular endothelial cell" collagen gel constriction was ameliorated by muscimol (γ-amino butylic acid (GABA)-A receptor agonist), baclofen (GABA-B receptor agonist), and eNOS gene ablations [64]. Amelioration of Hcy-induced endothelial cell collagen gel constriction may be induced by NO through GABA-A and GABA-B receptors.

Methionine-induced HHcy caused age-related impairment of cerebrovascular reactivity in healthy adults; this effect was ameliorated by treatment with quinapril, possibly due to improvement of endothelial NO-mediated vasodilatation [17]. Plasma Hcy levels both under basal conditions and post-methionine load were higher in patients with Alzheimer's disease (AD) and patients with vascular dementia than in normal controls; vitamin B_{12} basal levels negatively correlated with basal Hcy levels only in AD patients, suggesting the possible role of chronically elevated Hcy in neuronal degeneration in demented patients [84]. Clinical findings show that elevated Hcy levels in plasma are associated with decreasing levels of circulating endothelial progenitor cells (EPC) and that the Hcy-induced EPC reduction may be due to apoptosis through caspase-8-mediated release of cytochrome c; B vitamin (B_6 and B_9) intervention impairs Hcy-induced EPC apoptosis leading to increments in EPC populations [2].

Homocysteine, cerebral blood flow, and Alzheimer's disease

Supplementation of betaine, an Hcy metabolizer, ameliorated the Hcy-induced memory deficit and enhanced long-term potentiation and also decreased Aβ production in HHcy rats, possibly due to metabolism of Hcy [13]. Diet-induced high Hcy resulted in an exacerbation of memory deficits and Aβ and tau neuropathology in mice [47]. Diet-induced HHcy develops cerebral amyloid angiopathy via a reduction of Aβ clearance and transport within the brain in mice [46].

There is a significant increase in plasma concentration of Hcy and ADMA and a decrease of NO in plasma in AD patients, as compared with control subjects; the inhibition of endothelial NO synthesis by ADMA impairs cerebral blood flow, and the inhibition of nNOS by ADMA may cause cognitive dysfunction in AD [62]. It is suggested that there is a negative, significant relationship between blood and urine Hcy levels and between blood Hcy levels and Mini-Mental State Examination scores, suggesting that the reduced urinary excretion induces elevated Hcy in blood, resulting in cognitive dysfunctions [32]. As already stated, HHcy decreases cerebral blood flow possibly by impairment of eNOS actions and enhancement of NO degradation via oxidative stress. From studies on patients with AD and with normal cognitive function, Cankurtaran et al. [11] suggested that a decrease in antioxidants and an increase in oxidative damage induced by HHcy are linked to AD. Huang et al. [36] reported that cognitive performance in mild AD can be reflected by hypoperfusion of the temporo-parietal region and total Hcy level is an independent risk factor for rapid cognitive decline. Elevated Hcy in adults aged 80 years or older was suggested to contribute to increased AD-type pathology [35]. Behavioral and psychological symptoms of dementia seem to be associated with plasma Hcy concentration in AD-type dementia [91]. It was suggested that high-Hcy and low-folate levels correlate with increased risk of AD occurrence [65].

Vicious cycle in endothelial dysfunction, hypoperfusion, and amyloid-β peptide deposition

As already been mentioned, HHcy is a risk factor for impairing cerebrovascular endothelial function and interfering with NO-mediated cerebral blood flow. Brain and cerebral microvessels from eNOS-deficient mice had higher protein levels of amyloid-β-protein precursor (AβPP) and β-site AβPP cleaving enzyme 1 (BACE1), as well as BACE1 activity and Aβ, suggesting that endothelial NO plays an important role in modulating Aβ-protein precursor expression [3]. AβPP and $Aβ_{1–40}$ were increased in hippocampal tissue of eNOS-deficient mice as compared to wild-type mice; eNOS-deficient mice performed worse in a radial arm maze test of spatial learning and memory, as compared to wild-type mice

[4]. These authors suggest that NO/cyclic GMP pathway may be an important therapeutic target in preventing and treating mild cognitive impairment, as well as AD. Cerebral hypoperfusion triggers vascular deposition of peripherally applied human Aβ-42, which is shifted from vasculature to the parenchyma [27]. Reduced cerebral autoregulation is associated with increased amyloid deposition and increased white matter hypersensitivity volume in humans [10]. Aging- and lifestyle-associated damages of the brain microcirculation may affect Aβ clearance and perivascular drainage, promoting cerebrovascular Aβ deposition [42]. Increased amounts of Aβ impair endothelial function in cerebral blood vessels [20, 57]. Endothelial dysfunction causes a decreased cerebral blood flow and as a result increases the Aβ accumulation that in turn impairs NO bioavailability and promotes cerebral hypoperfusion (Fig. 2) [76]. A decreased cerebral glucose metabolism may precede the Aβ deposition and Aβ accumulation in turn leading to further decreases in the cerebral blood flow, closing a vicious cycle [56, 75]. Aβ-degrading enzymes, including neprilysin- and angiotensin-converting enzymes, may help to maintain cerebral perfusion by reducing the accumulation of Aβ in cerebral blood vessels [51].

Coronary circulation

In rats with methionine diet-induced HHcy, coronary arterial dilatation in response to increased intraluminal flow was abolished, possibly because of enhanced production of superoxide anions [82]. In HHcy rats and mice fed methionine in drinking water, superoxide produced by the p22phox subunit of NADPH oxidase reduces the ability of NO to regulate mitochondrial function in the myocardium [5]. In rat cardiac microvascular endothelial cells, Hcy increased inducible NO synthase and decreased endothelial NOS without altering neuronal NOS levels; there was accumulation of ADMA, possibly because of reduced DDAH expression [81]. In conscious dogs fed with methionine to increase plasma Hcy, veratrine-induced, NO-dependent coronary vasodilatation was reduced but was restored by infusion of ascorbic acid or apocinin; HHcy decreased bradykinin- or carbachol-induced reduction of myocardial oxygen consumption, and this effect was restored by co-incubation with ascorbic acid, tempol, or apocinin, suggesting that HHcy impairs NO bioavailability through oxidative stress [71]. In rats, pretreatment with folic acid blunted myocardial dysfunction during ischemia and ameliorated post-reperfusion injury. It was suggested that folic acid preserves high-energy phosphate and reduces subsequent reactive oxygen species generation, eNOS uncoupling, and post-reperfusion injuries [52]. BH_4 supplementation of murine endothelial cells revealed that calcium ionophore-evoked NO bioactivity correlates with intracellular BH_4/BH_2 and not with absolute intracellular BH_4; superoxide production negatively correlated with intracellular BH_4/BH_2, suggesting that diminished BH_4/BH_2 rather than BH_4 depletion per se is the trigger for NO insufficiency [21]. Folic acid administration to post-myocardial infarction in mice improved cardiac ejection fraction and induced tissue inhibitor of metalloproteinase, Hcy-metabolizing enzymes, and 5-methylene tetrahydrofolate reductase, suggesting that folic acid improves myocardial function in myocardial infarction [58]. Plasma HHcy induced by high-methionine diet in rats was prevented by treatment with palm tocotrienol-rich fraction; this compound also reversed the decreased glutathione peroxidase

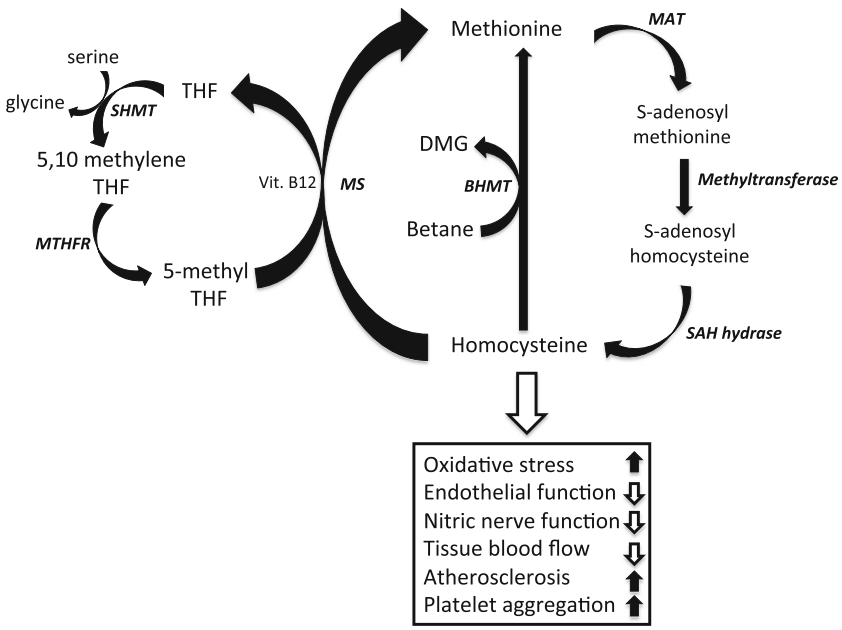

Fig. 2 Schematic overview of homocysteine metabolism and its relationship with folic acid and vitamin B_{12}. *THF* tetrahydrofolic acid, *SHMT* serine hydroxymethyl transferase, *MTHFR* methylene THF reductase, *MS* methionine synthase, *DMG* 5*N*,10*N*-dimethylglycine, *BHMT* betane homocysteine methyltransferase, *MAT* methionine adenosyltransferase

activity, suggesting that the palm extract is comparable to folate-reducing methionine diet-induced HHcy and oxidative stress [54].

Elevated serum Hcy is an established risk factor for myocardial infarction not only in men but also in middle-aged women [92]. Adenosine-stimulated myocardial blood flow increase was reduced in patients with HHcy, and flow-mediated brachial artery dilatation was also impaired, suggesting that acute HHcy impairs coronary microvascular dilatation as a result of reduced NO bioavailability [74]. In hyperhomocysteinemic patients with coronary artery disease who were treated with folic acid/cobalamin, coronary blood flow increased after ACh infusion, compared with a decrease in blood flow in the placebo-treated group, suggesting that coronary endothelial function improves after treatment with folic acid and cobalamin [88]. Hcy levels were positively correlated with endothelin-1 level and negatively correlated with NO/endothelin-1 in patients with coronary lesions [18]. Chronic HHcy impairs coronary endothelial function, and plasma levels of NO and BH_4 are positively correlated and significantly decreased in patients with HHcy compared with controls [41]. In patients with chronic HHcy, plasma levels of NO and BH_4 were lower and coronary flow velocity reserve was impaired as compared with the control group [33]. Tsuda and Nishio [79] suggested that decreased bioavailability of NO due to ADMA may partially explain the increased risk for coronary heart disease in women with HHcy.

Renal circulation

Incubation of isolated rat small renal arteries with Hcy attenuates the increase in NO in the renal arterial endothelium; decreased NO is not the only mechanism resulting in endothelial dysfunction but also increased superoxide levels in the arterial endothelium [48]. In HHcy rats, superoxide anion concentrations and nitrite + nitrate levels were higher, glomerular filtration rate was lower, renal plasma flow was lower, and renal vascular resistance was higher, as compared with control rats; after treatment with L-arginine, the responses of glomerular filtration rate, renal plasma flow, and renal vascular resistance were attenuated [29]. HHcy seems to induce oxidative stress, NO inactivation, and renal dysfunction involving disturbances in the NO pathway. The renal blood flow increase mediated via endothelium-dependent hyperpolarizing factor was also inhibited in HHcy rats [24]. Chien et al. [19] reported that in children with early chronic kidney disease, blood pressure abnormalities assessed by ambulatory blood pressure monitoring were associated with L-arginine-to-ADMA ratio, Hcy, and L-cysteine. These authors emphasized that the effect of NO and the Hcy pathway is important in the genesis of chronic kidney disease-related hypertension.

The glomerular filtration rate increased by diabetic induction in mice was normalized following treatment with the peroxisome proliferator-activated receptor-γ (PPARγ) agonist ciglitazone that ameliorated the decreased bioavailability of NO in diabetic mice [63]. Ciglitazone appears to protect against diabetic nephropathy by activating PPARγ and clearing glomerular tissue Hcy. Several lines of evidence suggest that PPARγ ligands protect endothelial function and improve vascular functions [40, 60, 66] Kidneys isolated from HHcy rats showed increased levels of superoxide anions and lipid peroxides; folic acid supplementation antagonized HHcy-induced oxidative stress via its HHcy-lowering effect, attenuated xanthine oxidase activity, and restored SOD activity in the kidney [38]. Folic acid appears to be effective in protecting kidneys against oxidative stress. Lowering plasma Hcy by folic acid inhibits transforming growth factor-β1 expression and attenuates HHcy-induced glomerular damage [12].

Betaine is the substrate of the kidney- and liver-specific betaine-Hcy methyltransferrase, an alternative pathway for Hcy remethylation. In the diet-induced HHcy rat model, betaine was decreased in all tissues analyzed (kidney, liver, and heart); in the mouse cystathionine β-synthase deficiency model, betaine was decreased in plasma, liver, heart, and brain but was conserved in kidney [39].

Others

When endothelial cells were exposed to Hcy or to its precursor methionine, ADMA concentration in the cell culture medium increased. Hcy-induced accumulation of ADMA was associated with reduced NO synthesis by endothelial cells and segments of pig aorta; Hcy reduced the activity of dimethylarginine dimethylaminohydrolase (DDAH), the enzyme that degrades ADMA [70]. Hcy appears to inhibit DDAH enzyme activity, causing ADMA to accumulate and inhibit NO synthesis.

In rats with diet-induced HHcy, the vasodilator response to ACh was attenuated, suggesting that Hcy might contribute to defective NO availability; Hcy-induced endothelial dysfunction is compensated by H_2S in a NO-independent manner [25]. These authors suggested that impaired vasodilatation, possibly via the eNOS inhibition and hepatic stellate cell contraction caused by Hcy, contributes to the dynamic component of portal hypertension. Hcy treatment increased blood pressure in wild-type mice, whereas in HHcy-cystathione β synthase heterozygote knockout ($CBS^{-/+}$) mice, Hcy did not increase blood pressure; DDAH-2 and eNOS expressions in mesenteric arteries were decreased in mesenteric arteries in $CBS^{-/+}$ mice compared with wild-type mice, suggesting that HHcy causes mesenteric remodeling and narrowing by decreasing eNOS expression [53]. The tibial bone blood flow and eNOS protein levels in HHcy-$CBS^{+/-}$ mice decreased, as

compared with those in wild-type mice; these effects in CBS mice were ameliorated by folic acid supplementation, suggesting the efficacy of folic acid on HHcy-induced bone loss [80]. There were increases in flow-mediated blood flow in control rat gracilis muscle venules, but constrictions were induced in HHcy rat; flow-induced constrictions seen in HHcy rats were converted to dilatations in the presence of thromboxane A_2-receptor antagonists, which were abolished by the NOS inhibitor L-NAME and the cyclooxygenase inhibitor indomethacin. A superoxide dismutase diminished flow-induced venular constrictions in HHcy rats, suggesting that TxA_2 and reactive oxygen species overcome NO and vasodilator prostaglandins in the venule circulation of HHcy rats [59].

It was suggested that elevated serum Hcy is responsible for the endothelial damage in Bahçet's disease and may be an additional risk factor for the development of retinal vascular occlusive disease [28]. Microparticles (MPs) are small membrane vesicles released by stimulated or apoptotic cells. Human umbilical vein endothelial cells stimulated by Hcy produced more microparticulates than umbilical endothelial cells under the control conditions; endothelial function impairment due to HHcy is related to microparticulate shedding, which may involve platelets and vascular cells [61].

Summary

In this review, we have discussed impaired regional blood flow in experimental animals and humans by HHcy in relation to suppressed function of NO produced in the endothelium and parasympathetic vasodilator neurons. Endothelial NO plays important roles in decreased vascular resistance and increased regional blood flow in the heart, kidney, and other peripheral organs and tissues, whereas nitrergic neuronal vasodilatation plays important roles in increasing cerebral blood flow. In cerebral arteries and arterioles, both endothelial NO and neuronal NO are involved in vasodilatation and blood flow increase; in renal vasculatures, endothelial NO and neuronal NO participate in vasodilatation, but adrenergic nerves play a role as vasoconstrictors via α-adrenoceptor stimulation. In coronary vasculatures, endothelial NO and norepinephrine as a β-agonist contribute to vasodilatation but neuronal NO does not appear to play a role as a vasodilator [77]. Cerebral hypoperfusion functionally induced via NO-related mechanisms elicits not only reduced blood supply but also increased production of β-amyloid, this substance interfering with brain circulation via reduced endothelial function [76]. This is in contrast to brain embolism chronically and histologically induced. These effects induced by increased plasma concentrations of Hcy are expected to be involved in the genesis and development of cognitive failure and AD. Impaired coronary blood flow by HHcy is one of the risk factors in eliciting anginal attack and myocardial infarction. Impaired circulation in the kidney and other organs and tissues by increased concentrations of Hcy not only participate in local blood flow impairment but also in increasing systemic blood pressure. Folic acid, vitamin B_6/B_{12}, and related substances decrease plasma concentrations of Hcy and expected to play roles in the prevention and treatment of circulatory diseases and AD. Increased HHcy is one of the risk factors for defective functional control of cerebral, coronary, renal, and other vasculatures. It would be important to recognize that HHcy-induced cerebral hypoperfustion due to impaired endothelial function triggers a vicious cycle of cerebral hypoperfusion-increased, β-amyloid deposition-induced endothelial dysfunction [76], leading to an incidence of cognitive failure and AD. Further efforts devoted to advance our understanding of physiological and pathophysiological actions of endothelial and neuronal NOs, and its counteracting molecules such as oxidative stress and ADMA on regional and systemic blood flow regulation will contribute to development of novel ways for prevention and therapy against cardiovascular disorders.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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