

A novel approach in the management of hyperhomocysteinemia

Shaiba Sana Qureshi^a, Jeetendra Kumar Gupta^{a,*}, Ahsas Goyal^a, Harlokesh Narayan Yadav^b

^a Department of Pharmacology, Institute of Pharmaceutical Research, GLA University, Mathura 281406, UP, India

^b All India Institute of Medical Sciences (AIIMS), New Delhi 110029, India



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ABSTRACT

Hyperhomocysteinemia (Hhcy) is a biochemical alteration with plasma levels of homocysteine higher than 15 $\mu\text{mol/L}$, associated with atherosclerosis, and with vascular thrombosis by disrupting endothelial cells. Homocysteine is a sulfur-containing amino acid derived from methionine which is an essential amino acid. Excess homocysteine produced in the body is expelled out by liver and kidney from the systemic circulation. Hhcy is caused by the excess deficiencies of the vitamins like pyridoxine (B6), folic acid (B9), or cyanocobalamin (B12). High protein consumers are usually at risk for hyperhomocysteinemia because of low plasma B12 levels. It is approximated that mild Hhcy occurs in 5–7% of the general population and 40% in patients with vascular disease. Patients with heart failure, impaired renal function, and diabetes should be screened since the prevalence of Hhcy in these patients appears to be quite high. In this article, we hypothesize that citicoline is a novel drug for the management of Hhcy. Furthermore, the side effects of citicoline are also minimal and self-limiting. If this strategy is validated, citicoline will be the cost-effective way to be administered for Hhcy. Many evidences are available which suggest that ignoring homocysteine levels in patients with the vascular disease would be unwise. Thus, there is an urgent need for health care providers to develop effective preventions and interventions program (folic acid, Vitamin B6 and Vitamin B12 supplementation as well as lifestyle change) to reduce this disorder.

Introduction

Hyperhomocysteinemia (Hhcy) is a biochemical alteration characterized by an abnormally increased level of homocysteine in the blood; above 15 $\mu\text{mol/L}$. Liver and kidney eliminate excess homocysteine from the blood. Hhcy induces blood clots in veins and arteries [1]. Bing reported that homocysteine is a sulfur-containing amino acid, isolated from a urinary bladder stone in 1933 by Vincent du Vigneaud [2]. It is metabolized either by remethylation pathway to methionine or the transsulfuration pathway to cysteine. The former path is dependent on the proper functioning of methionine synthetase, methylenetetrahydrofolate reductase enzyme, vitamin B12, and folic acid. The latter pathway is dependent on the enzyme's cystathionine beta-synthetase and methyltetrahydrofolate reductase [3]. Intracellular homocysteine is also released into blood and urine. Hhcy is a rare autosomal recessive disorder with severe elevations of homocysteine in urine and plasma [4].

The prevalence of Hhcy varies extensively with geography, sex, ethnicity, and age [5]. Carmel *et al.* demonstrated the lowest homocysteine concentration among Asian Americans, medium concentration among Hispanic Americans and maximum concentration among

Caucasians [6]. Refsum H *et al.* reported that homocysteine concentration is higher in men and the elderly [7]. Amouzou *et al.* said that coastal West Africa people had a higher prevalence of Hhcy than the people located in the interior part of countries [8]. It is approximated that mild Hhcy occurs in 5–7% of the general population and 40% in patients with vascular disease [9,10]. The incidence of Hhcy in American society is just 5–7%, in Chinese 27.5% and in Indians 52–84% [11,12]. Other workers reported that Indians are prone to higher homocysteine levels than Europeans [13]. In 1990–98, stroke mortality in the US was falling at 0.3% per year, but after folate substitution in 1998, the mortality dropped to 2.9% per year, 10 times change. The prevalence of homocystinuria in the United States is approximately 1 per 100,000 [14]. A mortality rate of 18 percent by the age of 30 has been reported from worldwide series of 629 patients with cystathionine beta synthase (CBS) enzyme deficiency [15]. The persistence of polymorphism (common mutation in methyl tetrahydrofolate reductase gene) is decidedly less in some residents (< 1% in African descent) and significantly high in others (11–15% in Anglo-Americans, > 20% in Italian and 25–57% in Mexican population) [16]. Bangladeshi men have a high prevalence of Hhcy which has been related to smoking and betel nut use [17].

* Corresponding author.

E-mail addresses: jkgupta81@rediffmail.com, jk.gupta@gla.ac.in (J.K. Gupta).

Folic acid, Vit B12, and Homocysteine are metabolically closely related, and there are several factors that cause elevation of homocysteine levels in the blood. They are as follows-

Lifestyle factors

Smoking is linked with vascular disease and associated complications related to Hhcy. The extent cigarettes smoked a day is the most vigorous root of Hhcy. Nicotine directly alters the methylation and catabolizes folate cycle. High alcohol utilization is also linked with a gastrointestinal disorder, which results in decreased absorption of vitamins and folic acid, thus contributing to increased homocysteine levels. It also inhibits methionine synthase enzyme to decrease hepatic uptake as well as increase excretion through urine [18].

Vitamin deficiency

Vitamins like (B6, B12, and folic acid) affects the enzymes involved in homocysteine metabolism. High protein consumers are often at risk for Hhcy. Coffee (4 cups/day) is associated with an increase in homocysteine level although this response can be overcome by supplementing with 200 mg/day of folic acid [1,19].

Enzyme deficiency

The metabolism of homocysteine is dependent upon methyl donor and several cofactors. Genetic defects in genes encoding for enzymes such as Methyl tetrahydrofolate and Methionine synthase causes the deficiency. These enzymes are involved in the homocysteine metabolic pathways. Methyltetrahydrofolate enzyme is responsible for the transformation of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (active folate which donates its methyl group to homocysteine to form methionine). Certain rare defects in this genetic makeup cause homocystinuria, brain damage and childhood cardiovascular disease [20].

Renal dysfunction

Renal failure patients have extremely high homocysteine levels due to its less efficient renal clearance. Patients with kidney disease have high rates of cardiovascular morbidity and mortality. Disproportionate level of plasma is a culprit for increase homocysteine levels. The underlying cause of Hhcy in kidney disease is not yet understood, although reduced plasma homocysteine clearance is the most immediate cause [21].

Drug interaction

Certain drugs such as cholestyramine and metformin prevent vitamin absorption from the gut. Methotrexate, nicotinic acid and folic acid derivatives interfere with folate and homocysteine metabolism. Oxcarbazepine and topiramate might cause Hhcy because of their capacity to activate hepatic enzymes [22,23].

Hhcy in mother increases the risk of birth defects, dementia, and bone fracture. Patients with this disease have 6 times greater mortality than patients with low homocysteine levels. The risk conferred by homocysteine multiplies the risk conferred by other factors such as smoking, diabetes, hypertension and lipid disorders. There are claims that increase in folic acid intake reduces 3 $\mu\text{mol/L}$ homocysteine and reduces the risk of ischemic heart disease by 16%, stroke by 24% and deep vein thrombosis by 25% [24,25]. Elevated plasma homocysteine is an independent risk factor for Cardiac impairment and Stress Hypoxia. A 5 $\mu\text{mol/L}$ elevation in homocysteine increases all-cause mortality by 49%, cancer mortality by 26%, cardiovascular mortality by 50%, and non-cancer, non-cardiovascular mortality by 104% [16]. The relationship between Hhcy and atherosclerosis was proposed by Mc Cully in

1969. It is now well recognized that Hhcy is a strong, independent risk factor for stroke, myocardial infarction and other vascular events [26]. Homocysteine is an unstable amino acid, which undergoes auto-oxidation to produce free oxygen radicals which further increases oxidative stress [27]. Bright A *et al.* reported that Hhcy contributes to atherosclerosis in two ways. One includes the generation of free oxygen radicals which converts low-density lipoproteins of subendothelial tissues to oxidized low-density lipoproteins. Oxidized LDL further acts as a key mediator of the inflammatory process in atherosclerosis. Oxidized LDL releases vascular cell adhesion molecule and monocyte chemoattractant protein. The monocytes then get reformed to macrophages, which take up Oxidized LDL to get converted to foam cells. The foam cells get settled below the endothelium to form fatty streak. The latter part includes the suppression of nitric oxide activation by free radicals, which results in endothelial dysfunction and contribute to atherosclerosis [28]. Hhcy has been associated with homocystinuria and many other diseases involving the CNS, such as stroke. Energy metabolism and oxidative stress are related to the pathogenesis of this disease. Energy demands of the central nervous system are supplemented with continuous supplies of oxygen and glucose through the blood flow. Increased homocysteine level reduces ATP availability for neurons and causes severe neuronal injury. The disrupted metabolism of homocysteine is associated mainly with tissue hypoxia and vascular disease (Fig. 1) [29].

Methodology

In this study, a hypothesis is created for the management of Hhcy. The first step of this study involves planning about the metabolism and mobilization of homocysteine. On the basis of which utility of citicoline is hypothesized in the latter half. Hence, the basic elements of this study can be characterized as follows:

- Pathway for mobilization of homocysteine
- Design of hypothesis

Pathway for mobilization of Homocysteine

Mammals, in comparison to bacteria and plants, cannot make their own methionine. Thus, in humans and animals, methionine is a necessary amino acid which is provided in the form of proteins ingested as food. Fig. 2 depicts that in digestive tract dietary proteins are hydrolyzed to amino acids. Methionine liberated from dietary proteins is taken up by the epithelium of the gastrointestinal tract and transported in the blood to cells of various organs. Homocysteine is a sulfur-containing amino acid which is produced from the metabolism of methionine, the metabolism of which involves four steps. The first step is the transmethylation pathway in which the conversion of methionine to homocysteine takes place. This pathway consists of the formation of (S-adenosylmethionine) which transfers a methyl group to a number of several methyls acceptor molecules (proteins, DNA, neurotransmitters) and forms adenosylhomocysteine, which is further converted into homocysteine. The second step is the trans-sulphuration pathway which involves the permanent conversion of homocysteine to cysteine with the help of cystathionine- β -synthase (CBS) and pyridoxine as an essential cofactor. Remethylation pathway is the third step in which formation of methionine takes place from homocysteine by methionine synthase enzyme along with 5, 10-methylenetetrahydrofolate (MTHF) and Vitamin B12 as essential cofactors. Regeneration of methylenetetrahydrofolate (5-MTHF) from tetrahydrofolate (THF) via 5,10-methylenetetrahydrofolate reductase enzyme is the last step in the metabolic pathway [30].

Design of Hypothesis

We propose Citicoline, a mononucleotide to show an enhanced

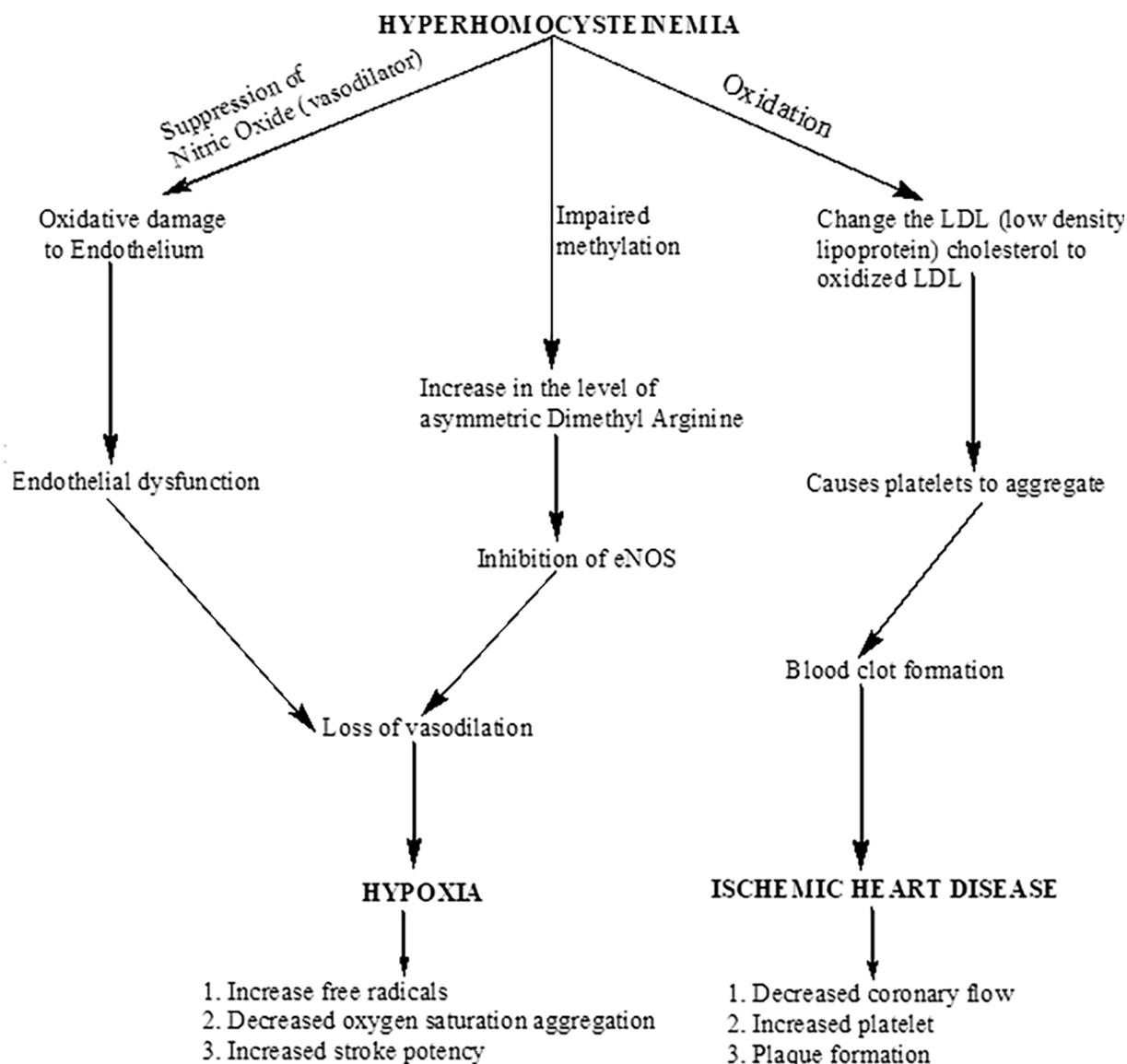


Fig. 1. Hyperhomocysteinemia induces hypoxia and cardiac impairment.

effect for the treatment of Hhcy. We suggest the following:

Citicoline or cytidine diphosphocholine is a mononucleotide which is composed of cytosine, ribose, choline, and pyrophosphate. It is a necessary intermediate in the synthesis of cell membrane phospholipid mainly phosphatidylcholine. Citicoline was identified in 1955 and synthesized in 1956. It is an exogenic source of acetylcholine (a key neurotransmitter). It's been studied in Europe, USA, and Japan for many years. It is extensively available for the treatment of neurological disorders. Citicoline is offered as a nutritional supplement in the United States of America. It has many effective mechanisms of action leading to beneficial effects on neurological function. Citicoline primarily acts via increasing the synthesis of phosphatidylcholine which further raises the production of acetylcholine. Absorption by the oral route is nearly complete, and bioavailability is almost the same as by the intravenous route. Once absorbed it is widely distributed in the body, crosses the BBB and reaches the central nervous system, where it is integrated into the membrane and microsomal phospholipid fraction. It triggers the biosynthesis of structural phospholipids of neuronal membrane elevate brain metabolism and affect the level of neurotransmitters. Oral administration of citicoline increases the plasma level of choline and cytidine (building blocks which are used to revive neuronal membrane integrity). Further, it reduces the deposition of free fatty acids,

arachidonic acid, toxic metabolites like prostaglandins and thromboxanes, restoring membrane function at the site of lesion and attenuating free radical damage. Citicoline decreases norepinephrine and increases dopamine in the condition of cerebral hypoxia. It may increase the dilation of blood vessels in animals with cerebral microcirculation injury, increasing cerebral blood flow. It is hydrolyzed in the intestinal tract to form choline and cytidine (nucleoside of cytosine) to generate phospholipid. Choline preferentially used for acetylcholine synthesis while cytidine for various nucleotide. Choline in small amount is produced in the brain. Because of its low production, it's taken into consideration as an essential nutrient and plays several roles in human physiology. Citicoline is a safe drug which has no adverse effects even at 2000 mcg per day in human volunteers during phase II and phase III clinical trials. It is a well-tolerated drug [31–33] Fig. 3.

In this article, we hypothesize that citicoline is a novel method for treating Hhcy. Choline derived from citicoline synthesizes betaine (methyl donor) via choline oxidase enzyme, which provides methyl group for the conversion of homocysteine to methionine via BHMT (Betaine-homocysteine methyltransferase enzyme). If this strategy is validated, citicoline will be a cost-effective way to be administered for Hhcy.

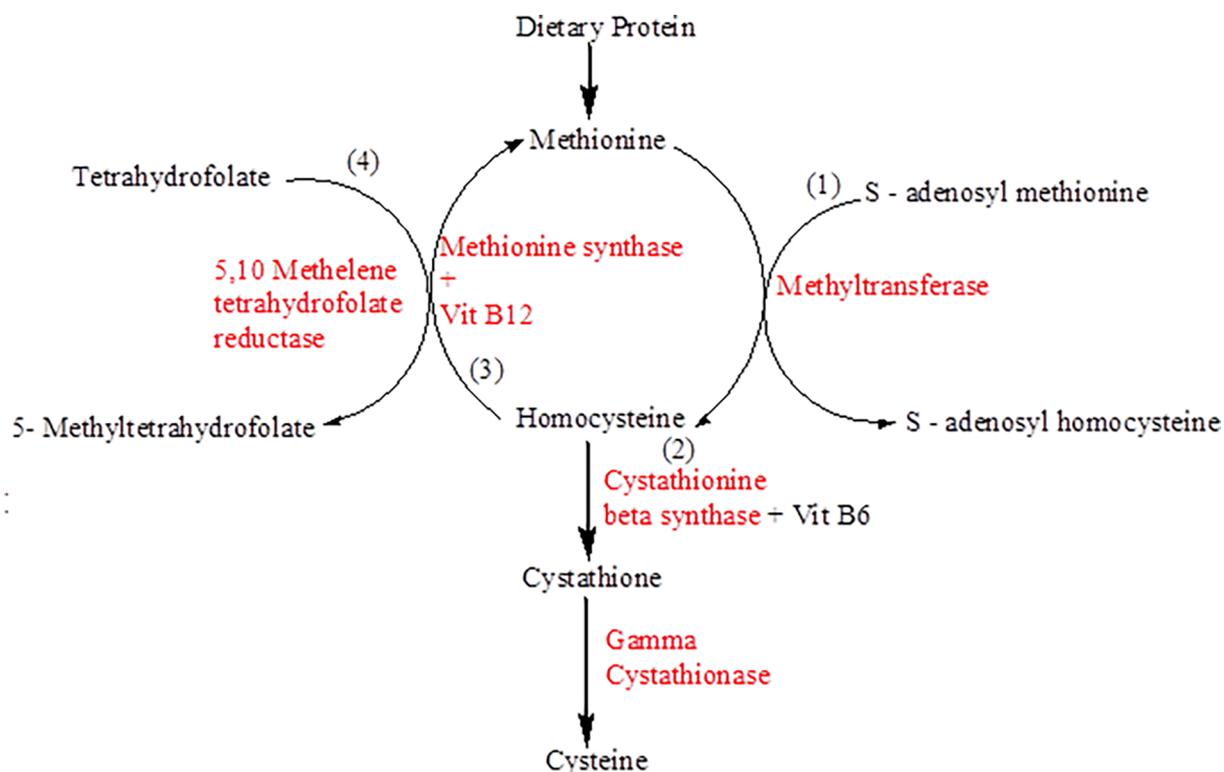


Fig. 2. Diagrammatic representation of the metabolic pathway of homocysteine.

Discussion

A novel approach to treat Hhcy with citicoline has been discussed in this article. Hhcy is a pathological condition with plasma levels of homocysteine higher than 15 μmol/L, associated with atherosclerosis, and with venous and arterial thrombosis by damaging endothelial cells.

The Internationally confirmed treatment for Hhcy involves the use of folic acid, vitamin B12, and pyridoxine. Folic acid and vitamin chiefly act under a fasting condition, and pyridoxine acts after meals. Pyridoxine reduces homocysteine levels by 22%. Folic acid alone reduces homocysteine level by 22% and vitamin B12 by 11%. When both are administered together, it causes a minimization of 38.5% [34].

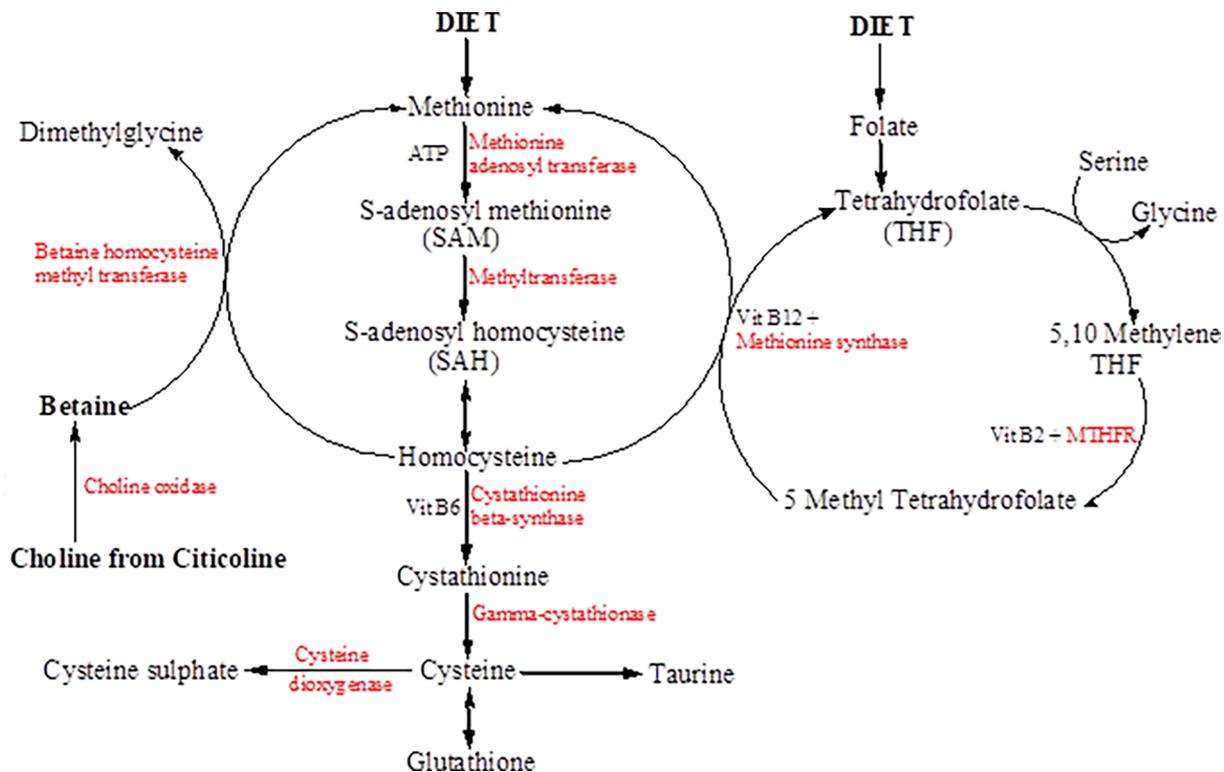


Fig. 3. Diagrammatic representation of hypothesis.

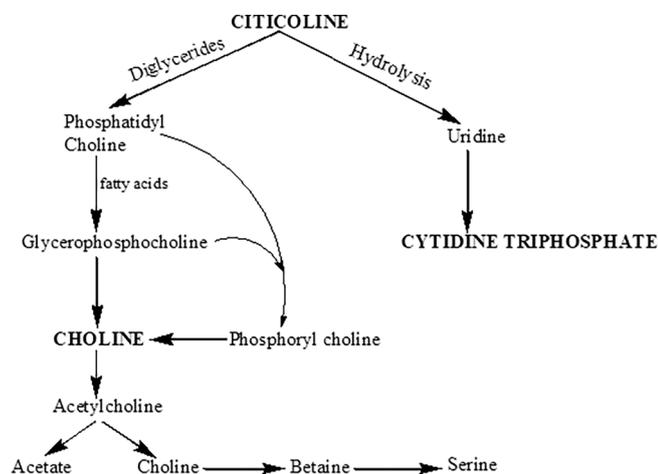


Fig. 4. Diagrammatic representation of mobilization of citicoline.

Montero *et al.* demonstrated that pyridoxine (600 mg/d) and folic acid (10 mg/d) when given for one month to the patients, a partial decline in homocysteine level was observed in some of the cases, but when betaine was added (6 g/d) in the dose, Hhcy disappeared [35]. Treatment also includes anticoagulant medications such as aspirin, clopidogrel, heparin, warfarin which prevent blood clots. Auer *et al.* suggested that small doses of folate (0.4–5 mg/d), vitamin B12 (400 µg/d) and vitamin B6 (10–50 mg/d) rapidly decrease homocysteine concentration. The major potential risk of vitamin B6 is sensory peripheral neuropathy with use over the years at 400 mg daily dose. He also reported that a combination of folic acid 25 mg, vitamin B6 25 mg and vitamin B12 250 µg/day reduces atherosclerosis, as estimated by carotid plaque area [36]. So, Citicoline can help in treating this disorder by decreasing excess homocysteine into amino acids via the BHMT enzyme (Fig. 4).

It provides the brain with cytidine and choline and supports the synthesis of betaine and acetylcholine, which is an adequate way for the treatment of hyperhomocysteinemia [31,33]. Citicoline is a new nutrient with many benefits for neurological dysfunction. It is an essential intermediate in the synthesis of phosphatidylcholine and plays numerous crucial roles in human physiology, i.e. cell membrane signaling. It attenuates the production of free radicals in ischemic condition. It improves cognitive impairment in Parkinson's and Alzheimer's disease, as well as in victims of moderate stroke and cerebral ischemia. It also reduces the prolongation of coma and motor deficits associated with traumatic head injuries. Citicoline is highly bioavailable with oral dosing. Based on data from clinical trials, its effective dosing ranges from 500 mg to 2000 mg per day. It is safe in pediatric and adult populations. Hhcy is a disorder worth treating. Over the past decade, homocysteine-related research provokes a massive amount of scientific literature and sparked vigorous debate, as an emerging risk factor for neural tube defects (NTD) and non-communicable disease (NCDs), including type 2 diabetes and cancer.

Conclusion

As it is evident in the mobilization pathway (Fig. 2), a little disturbance in the metabolism of homocysteine can lead to Hhcy. It can be considered a silent killer since initially, it is asymptomatic and after a long-time span becomes a big harpoon of morbidity and mortality. This study focuses on a hypothesis which aims to manage the elevated level of homocysteine via citicoline. Citicoline is a novel drug with many benefits for neurological dysfunction. When administered, citicoline is hydrolyzed in the intestinal tract to form choline and cytidine. Choline derived from citicoline synthesizes betaine (methyl donor) via choline oxidase enzyme, which provides methyl group for the conversion of homocysteine to methionine via BHMT enzyme and regulates the

metabolism of homocysteine.

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Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2019.109245>.

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