ADVANTAGES OF HOMOCYSTEINE INDUCED PARKINSON MODEL

Abstract

Homocysteine; a homologue of the amino acid cysteine is a non-proteinogenic sulphur-containing amino acid. Elevated levels of plasma homocysteine has been reported in PD patients undertaking a treatment of L-DOPA (L-3,4-dihydroxyphenylalanine).The methylation of L-DOPA by catechol-o-methyl transferase is reported as the leading cause of the elevated homocysteine levels in blood plasma in PD patients. The hypothesized mechanisms of neuronal damage by hypehomocystemia appear to be multiple and often overlapping. Reports from various authors has given a conclusion that nervous system might be particularly sensitive to extracellular homocysteine due to its excitotoxic properties.

Keywords: Homocysteine; PD model; Cysteine; Neurodegeneration; L-Dopa

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I. INTRODUCTION

Homocysteine; a homologue of the amino acid cysteine is a non-proteinogenic sulphur-containing amino acid. It is a natural byproduct of one-carbon metabolism like the methionine metabolism (Bonetti et al, 2016 & Nuru et al, 2018). Hcy is acted upon by the cofactors like vitamin B12 and and folate and is coverted into methionine. Similarly, methionine can be converted to homocysteine too (Bo Young et al, 2022). Hcy is not present in the diet but high methionine in our diet can lead to hyperhomocystemia as a result of the metabolism (Nuru et al, 2018). Elevated levels of plasma homocysteine has been reported in PD patients undertaking a treatment of L-DOPA(L-3,4-dihydroxyphenylalanine). The methylation of L-DOPA by catechol-o-methyl transferase is reported as the leading cause of the elevated homocysteine levels in blood plasma in PD patients (N. Bhattacharjee et al, 2016). Hyperhomocystemia have been reported to be one of the major cause of several diseases like bone metabolism derangements, cancer, thrombosis and neurodegeneration (Ansari et al, 2014). Homocysteine may also produce oxidative damage to enzyme proteins through the peroxides it creates during its metabolism (Olszewski et al, 1993). PD like symptoms are reported in many studies due to hypehomocystemia and a tryst have already developed to establish a new HCY induced Parkinson model.

II. BIOSYNTHESIS OF HOMOCYSTEINE

Homocysteine is a nonproteogenic sulfurated amino acid derived from the methionine rich diet one intakes. Homocysteine is not a direct component of the diet, but it is an intermediate in methionine metabolism. Each compound, methionine or homocysteine, is actually the precursor molecule for the other (Nuru et al; 2018). Some reports have stated homocysteine as the intersection of two pathways: remethylation to methionine, which is depenedent on folate and vitamin B12 (or betaine in an alternative reaction); and transsulfuration to cystathionine, which is pyridoxal-50 phosphate dependent (Bhatia et al, 2015). In the remethylation pathway, homocysteine takes up a methyl group from N-5-methyltetrahydrofolate thus forming methionine. A similar reaction with betaine as the methyl group donor has been discovered lately. The reaction with N-5-methyltetrahydrofolate can occur in most of the mammalian tissues and is highly dependent on vitamin B12, whereas the reaction with betaine is mainly found in the liver tissues and is not dependent on the levels of vitamin B12 (J. Selhub, 1999). SAM (S-adenosylmethionine) serves primarily as a universal methyl donor to a variety of acceptors. It is formed by the activation of methionine by ATP. S-adenosyl homocysteine (SAH), the by-product of these methylation reactions, is subsequently hydrolyzed to regenerate homocysteine. Another pathway of Homocysteine metabolism is the trans-sulfuration to cysteine with the help of the enzyme’s cystathionine-b-synthase and cystathionine-c-lyase and vitamin B6 as a cofactor as the cofactors. In this pathway, the homocysteine conjugates with serine forming the cystathionine. This occurs through an irreversible reaction which catalyzed by cystathionine β-synthase. Cystathionine is later hydrolyzed by γ -cystathionase to form cysteine and α-ketobutyrate (Mudd SH et al; 1980). The Excess cysteine maybe oxidized to taurine or excreted through the urine. Thus, in addition to the synthesis of cysteine, this pathway catabolizes the excess homocysteine not required for methyl transfer (Obeid R. et al; 2006). The SAM acts as one of the allosteric inhibitor of the enzyme methylenetetrahydrofolate reductase (MTHFR) and as an activator of the enzyme cystathionine β-synthase. Thus, SAM has the capacity to suppress the synthesis of N-5-methyltetrahydrofolate required for remethylation and promotes the initial reactions of transsulfuration cystathionine synthesis. Therefore, intracellular SAM concentration is an important determinant of the fate of homocysteine molecules (Van der Put N.M et al; 1998).

III. CAUSES OF HYPERHOMOCYSTEMIA

Hyperhomocystemia can be defined as a condition where the homocysteine levels are higher than the stipulated levels. The average plasma Hcy level range in human is 5–10 μM. Above this 16–30 μM results in moderate hyperhomocystemia, 31–100 μM leads to intermediate hyperhomocystemia and severe hyperhomocysteinemiais caused when the levels are higher than 100 μM(Hansrani, Gillespie, & Stansby et al; 2006). Homocystinuria are the resultant metabolic disorders, which actually leads to severe HHcy condition because of cystathionine-β-synthase (CBS) deficiency (Ganguly & Alam, 2015). Apart from this the absence of folic acid, vitamin B6, vitamin B12, and genetic mutation in methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MS), CBS, and cystathionine-γ-lyase (CSE) are also responsible for the increase in the Hcy level (Kamat et al., 2014).

1. **Dietary regulation of homocysteine:** Excessive consumption of methionine-rich foods like nuts, beef, lamb, cheese, turkey, pork, fish, shellfish, soy, eggs, dairy, and beans exceeds the daily intake of required methionie i.e 56 grams for male and 46 grams for female. Remethylation to methionine from homocysteine requires folate. So, lower intake of folate and vitamins like vit D and higher intake of methionine in diet is a major factor for the elevated homocysteine levels in our body (Hainsworth et al; 2016 & Debreceni b. et al; 2014).The increase in methionine intake increases the homocysteine levels in serum e. Remethylation to methionine from homocysteine requires folate. So, lower intake of folate and vitamins like vit D and higher intake of methionine in diet is a major factor for the elevated homocysteine levels in our body (Hainsworth et al; 2016 & Debreceni b. et al; 2014).

2. **Biosynthetic regulation of homocysteine levels:** The levels of SAM synthetase (S-adenosyl-methionine synthetase) and AHCY (S-adenosyl-homocysteine hydrolase) regulates the homocysteine levels of our body. S-adenosylmethionine (SAM) is a universal methyl donor obtained by the methylation by S-adenosyl-methionine synthetase and then demethylated to S-adenosylhomocysteine (SAH). The demethylated SAH finally transformed to Hcy by AHCY (Brattstrom L. et al; 1994). Thus, higher the levels of SAM synthetase and AHCY higher wil be homocysteine levels. Excess of betaine-homocysteine methyltransferase, cystathionine β-synthase – CBS and cystathionine γ-lyase will lead to decreased homocysteine levels. Homocysteine has two pathway i.e it can remethylated to Met or it can be trans-sulfurated to cysteine (Ostrakhovitch EA. et al; 2015). The levels of s-adenosylmethionine synthetase and s-adenosylhomocyteine hydrolase are directly proportional to the homocysteine levels and the enzymes like betaine-homocysteine methyltransferase and CBS are inversely proportional to the homocysteine levels (Chiku et al;2009). Other enzymes like the MTHFR (5,10-Methylenetetrahydrofolate reductase) and MARS or AARS, methionyl-tRNA synthase regulates the homocysteine levels too (Natalie C. Chen et al; 2010).

3. **Fluctuations in homocysteine levels due to concomitant diseases:** Patients with renal failure have been reported to have high levels of homocysteine. Although the exact

regulatory connection is not yet understood , but it has been reported that diseases like renal failure, gastric atrophy and inflammatory bowel disease increases the levels of homocysteine. Some authors reported that the Hcy metabolic capability is enhanced in both Type 1 and Type 2 diabetes in preclinical models probably due to a major kidney metabolic contribution (via increased trans-sulfuration pathway activity in both and in the second one probably betaine-Hcy methyltransferase too) (Van Guldener C; 2016).

4. **Genetic regulation of homocysteine levels:** Genetic alteration results in decreased expression of Hcy metabolizing enzymes, viz. CBS, MTHFR, and methionine synthase (MS), leads to hyperhomocysteinemia. MTHFR is the rate-limiting enzyme in the methyl cycle of homocysteine metabolism and it is encoded by the MTHFR gene (Lea R. et al; 2009). High homocysteine level has been reported in individuals with MTHFR C677T genotype as compared to the normal MTHFR C677C genotype (Goyette P. et al; 1994). The *MTHFR* C677T gene mutation results in a valine instead of an alanine. A second polymorphism that seems to have sufficient impact on enzyme activity to have possible clinical relevance is A1298C resulting in an alanine instead of a glutamate (Wu YL et al; 2013).

5. **Effects of hyperhomocystemia:** Hyperhomocysteinemia is associated with a wide range of clinical manifestations, mostly affecting the central nervous system (e.g., mental retardation, cerebral atrophy and epileptic seizures). Apart from all these hyperhomocysteinemia can be linked with an increased risk for atherosclerotic and thrombotic vascular diseases (Temple ME et al;2000). The most reported mechanism regarding pathogenesis of hyperhomocystemia is related to the oxidative stress.

IV. EFFECT OF HOMOCYSTEINE ON NEURODEGENERATION

The hypothesized mechanisms of neuronal damage by hypehomocystemia appear to be multiple and often overlapping. Results of numerous authors lead to a conclusion that nervous system might be particularly sensitive to extracellular homocysteine due to its excitotoxic properties (Thompson GA et al; 1996). The excitotoxic properties of homocysteine and its derivatives stimulates a subtype of glutamate receptor-NMDA receptors which acts as an antagonist and damages the neuronal DNA. This increases the susceptibility to apoptotic cell death (Kruman II et al; 2000). according to Sharma M et al 2015 there are some other factors or mechanisms through which hyperhomocystemia can lead to the neurodegenerative diseases. Those factors can be cited as Oxidative stress, mitochondrial ETS impairment, lipid peroxidation, thrombosis, amyloid-beta (Aβ) deposition and predisposition of *N*-homocysteinylated proteins epigenetic modifications Hyperhomocystemia is reported to increase the brain permeability. It is linked to increased MMP-9 and MMP-2 activity and the suppressed tissue inhibitors of metalloproteinase (TIMPs) (Yong VW et al; 2001). These in turn leads to the degradation of extracellular matrix (ECM). Eventually the blood-brain-barrier is destroyed.

1. **Homocysteine on age-related disorders:** Alzheimer’s disease (AD) is the most common neurodegenerative pathology that is responsible for significant mortality (Carly Oboudiyat et al; 2013). Age is one of the strong factors for Alzheimer’s disease (Plassman BL et al; 2010). The experiments all over the world indicates that oxidative stress plays a key role in Alzheimer's disease pathophysiology. The oxidative stress leads

to β-amyloid cleavage which results in neuroinflammation. As reported by Chai *et al.* elevated levels Hcy induces Aβ peptide accumulation. Aβ-peptides originates from the cleavage of precursor protein (APP) by the enzyme secretases. Clearance of those peptides actually triggers neurodegeneration (Hardy J et al; 2002). In AD and also results in the increase of AD-like tau hyperphosphorylation (PK Kamat et al; 2017). Some authors reported that administration of L-methionine in rodents produces a significant degree of VaD. Others have stated that intracerebral Hcy injections in rodent brains can produce AD like symptoms. Some reports have stated a transgenic mouse model of CBS (Cystathione-β synthase) induces HHcy leading to Aβ toxicity (PK Kamat et al; 2017). And this mechanism links Homocysteine to Aβ induced hippocampal neurotoxicity which is a potential source of neurodegenerative disease like alzheimer's disease. PD is the second most prevalent progressive neurodegenerative disorder after alzheimer's (Payam Saadat et al; 2018). Aging increases homocysteine levels and this seems to be related to the mild cognitive impairment associated to PD (Irizarry MC et al; 2005). According to the reports of B. Kocer et al; 2016 the folate and B12 deficiency in our diets with increasing levels of homocysteine leads to the atrophy of the neurons in the hippocampal regions with disruptions of cognitive processes. Some laboratories working on PD across the world have published that the levels of homocysteine is higher in the cerebrospinal fluids of the PD patients (C Isobe et al; 2005). There are reports suggesting the damage of MPTP-dependent (1-methyl,4-phenyl-1,2,5, and 6 tetrahydropyridine) dopaminergic cells by hyperhomocystemia (Suilleabhain et al; 2006 & Nivedita Bhattacharjee et al; 2016). The studies of Nivedita Bhattacharjee et al were directed towards the involvement of oxidative stress as a mechanism for the Hcy-induced dopaminergic neurotoxicity in mice. The complete experiment was designed to study the long-term effect of Hcy treatment on the motor behavioral activities in murine models, striatal dopamine levels and nigrostriatal levels of enzymatic (SOD, superoxide dismutase and catalase) as well as non-enzymatic (reduced glutathione) as the parameters of oxidative stress. The study suggested that long term administration of i.p injections of Hcy in mice might lead to motor behavioral deficits similar to PD. Moreover, homocysteine laeds to depletion of striatal dopamine and decreasing activity of nigral mitochondrial complex-I (Nivedita Bhattacharjee et al; 2016).

2. **Homocysteine on learning:** Many studies have reported that in acute homocysteine treatment the BDNF levels in the hippocampus of rats are reduced. And BDNFα is important to life maintenance and the memory processes (Matte C et al; 2009 & Matsumoto T et al; 2008).

3. **Hyperhomocystemia on depression like behavior:** Depression is the highest prevalent reversible neuropsychiatric disorder in the present-day world. Depression is actually a characteristic name for disinterest in activities, unusual fatigue and difficulty in concentrating and performing daily life activities due to excessive agitation (Reynolds E.H; 2013). The human MTHFR gene that is present in the 1p36.3 region of chromosome is responsible for the catalysis of (NADPH)-dependent 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Liu A. et al; 2010). The MTHFR gene mutation thus prevents the re-methylation of homocysteinen to methionine (Miller A.L. et al; 2008) . This leads to deficiency in folate and an increased level of homocysteine. And this results in various phenomenon that leads to the progression of the neurodegenerative diseases such as depression. According to some reports the molecular mechanism of depression might be

linked to DNA damage due to hyperhomocystemia and hypomethylation which in turn can lead to the death of neuronal cells (Narayan S.K. et al; 2014).

4. **Homocysteine on other diseases:** Hyperhomocystemia have been related to many inflammatory defects. The most direct effect of homocysteine levels is seen on the neurodegeneration as it acts as a neurotransmitter. It acts an agonist on the glutamate receptors (NMDA subtype) (Carmel R M et al; 2014). Apart from this homocysteine level alterations can cause CVD. CVD; cardiovascular diseases entirely comprises of the defects of heart and blood vessels (Mangge H et al; 2014). There are many factors that contributes to cardio vascular diseases but homocystemia have been stated as one of the major causes related to it (Shenoy V et al; 2014). A 40- fold increase of homocysteine can lead to a sudden infarctory stroke in adults of even less than 30 years. Reports by Ganguly et al; 2015 suggested that MTHFR mutations might lead to a pre-mature cardiovascular disease. Homocysteine mediated cardiovascular diseases might occur through several mechanisms. Some of the most studied mechanisms include- increased proliferation of smooth muscle cells of the vascular region, endothelial dysfunction, increased synthesis of collagen proteins which deteriorates the wall plasticity of the circulatory vessels(Zhang et al; 2000). Hyperhomocystemia have been related to kidney pathogenesis in many reports too. Kidney functions as one of the main sites of homocysteine metabolism and so high levels of accumulated homocysteine leads to severe chronic kidney diseases (J.D. House et al; 1998). Homocysteine accumulation leads to oxidative stress in the kidney, which antagonizes the vasodilator properties of nitric oxide through S-nitroso Hcy-formation. Homocysteine also decreases the SOD levels. This in turn leads to glomerulosclerosis and hemodynamic kidney dysfunction which changes the podocytes. Many other inflammatory diseases are linked to the hyperhomocystemia.

V. POSSIBILITY OF A HOMOCYSTEINE INDUCED PARKINSONIAN MODEL

Parkinson disease is a highly prevalent age-related neurological disorder after the Alzheimer's disease. The mechanism of neurodegeneration in Parkinson's disease (PD) remains elusive till now but many evidences have suggested that inflammation-derived oxidative stress plays a major role in the neurodegenerative pathway (Hirsch EC et al; 2009). Some researchers suggest that the loss of dopaminergic neurons in the substantia nigra and degeneration of pars compacta are hallmarks of the PD pathology. Apart from these the lewy bodies found in the brain is another specific hallmark of the degenerations concerning parkinsonian disorder. Some behavioral symptoms present in Parkinson disease are bradykinesia (akinesia in mice), rigidity and resting tremor (R.M. Camicioli et al; 2009). Thus, dopamine replacement therapy is used as a treatment to alleviate the motor symptoms of PD (Rajib Paul et al; 2016). Cognitive dysfunction and dementia are common outcomes in PD. Several studies have reported elevated Hcy levels in the plasma of PD patients compared to the healthy individuals (G. Gorgone et al; 2012). So, L-DOPA the precursor molecule of dopamine has been used as a treatment for ameliorating the PD like symptoms all over the world. But Levodopa termed as the gold-standard drug of PD across the world is known to increase blood homocysteine levels in PD patients (H.J. Kim et al; 2017). L-DOPA-induced elevated level of homocysteine is also evident in cerebrospinal fluid and in the nervous tissues of the brain (Joakim M. Tedroff; 1992). So, the present review has been written to study if a Parkinsonian murine model for study can be developed by administering homocysteine consecutively for a long term like 30 days or 60 days.

1. **Effect of homocysteine administration on the hallmarks of a parkinsonian model:** Effect of chronic homocysteine exposure on the behavior of mice models Various reports have claimed that administration of homocysteine in rats and mice causes notable motor behavioral changes (Eun-Sook Y. Lee et al; 2004). Nivedita et al have reported their findings that high doses of homocysteine(250mg/kg) for a period of 60 days have made mice highly akinetic in comparison to the controls. Apart from akinesia, catalepsy was also profoundly studied and was found that the mice showed cataleptic behavior even at lower doses when administered for long term (Nivedita et al; 2016). Other behavioral parameters have also shown changes as an effect of hyperhomocystemia such as learning and depression. Thus, long term homocysteine exposure shows behavioral deficits similar to the parkinsonian pathology.

2. **Effect of chronic homocysteine exposure on dopamine levels:** Homocysteine was reported to decrease the dopamine turnover upto 70% in the nigro-striatal regions of the brain. No such specific changes have been found in the hippocampal and cortex regions of the rat brain (Mattson et al; 2003). With the prolonged systemic administration of homocysteine at a high dose like 250g/kg there has been high depletion of dopamine levels. The striatal dopamine was reported to be decreased by 21% on the systemic administration of homocysteine on murine models (Nivedita et al; 2016). Thus, homocysteine administration shows similar dopamine depletion as it is found in the already established parkinsonian models. Although the exact molecular mechanism of depletion dopamine containing neurons in PD is not known clearly, however, oxidative stress has been postulated as one of the major factors. Similarly, homocysteine also depletes the dopamine levels through the elevation of oxidative stress.

3. **Effect of homocysteine on the mitochondrial complexes:** Mitochondrial dysfunction is one of the vital factors of various diseases in humans due to their important roles in cellular metabolism (Shigenaga et al; 1993). Some studies showed that homocysteine increases the mitochondrial pSTAT3 and also the oxidative stress in the cells. Such results have thrown a light in the darkest directions and indicated that oxidative stress and the overactivation of the mitochondrial STAT3 plays the main role in homocysteine induced mitochondrial injury in the hippocampal and the cortical regions of the brain (Shuang Chen et al; 2017). A significant decrease in the mitochondrial complex I activity is reported with the administration of homocysteine at higher levels (Nivedita et al; 2016).

4. **Effect of homocysteine on achetylcholine levels of the brain:** Hyperhomocysteinemia is associated with impaired acetylcholine levels in the brain. There has been a contradiction regarding the acetylcholinesterase activity regarding the effect on homocystemia. Many reports have suggested that acetylcholinesterase activity is increased on hyperhomocystemia (Renee M. Smith et al; 2019).

5. **Future perspective:** Models of parkinsonian or hemiparkinsonian disorders can be established with the systemic administration of pure homocysteine for a certain period of time. Shorter time points than the already studied 60 days' time periods can be done. This could be a better alternative than using the already established MPTP model as usage of

less harmful chemicals are safe for researchers and due to its prolonged deposition due to a longer half-life this is more stable too.

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