Hypothesis

Possible Treatment Concepts for the Levodopa-Related Hyperhomocysteinemia

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The saga of harmful levodopa (LD) in the treatment of Parkinson’s disease (PD) resulted from outcomes of animal—and cell culture studies and the clinical observation of motor complication related to the short half life of LD. Further aspects of LD long term application, the LD associated homocysteine increase and its emerging consequences on progression, and onset of neuropsychiatric symptoms and of vascular disease are only partially considered. Therapeutic approaches for this LD-mediated neurotoxic homocysteine increase are vitamin supplementation or LD application with an inhibitor of catechol-O-methyltransferase (COMT). However, forcing central dopamine metabolism further down the methylation path by central blocking of COMT and MAO-B may reduce oxidative stress and homocysteine levels. But it may also increase N-methylation of tetrahydroisoquinolines to neurotoxic N-methylated tetrahydroisoquinolines. These compounds were observed in cerebrospinal fluid and plasma of long term LD-treated PD patients. Therefore LD application with peripheral COMT inhibition may be safer.

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1. Introduction

The discussion of the value of levodopa (LD) for treatment of patients with Parkinson’s disease (PD) focused on a putative LD toxicity to date. This saga of harmful LD based on outcomes of animal—and cell culture studies in basic research and the clinical observation of the manifestation of long term motor complications. Therefore the Earlier versus Later L-DOPA (ELLDOPA) trial was designed to answer whether LD is harmful or not. The outcomes showed that LD is an efficacious and well-tolerated compound for treatment of PD patients. This study also provided also some evidence for progression modifying benefits of LD in PD patients, since—after a two wash out period of LD at the end of the trial—the PD patients with prior LD treatment performed significant better than the ones on placebo. One may assume that LD treatment maintained body function and prevented onset of secondary long term changes and adaptation of the body to the manifestation of PD. This may support the concept of an early diagnosis of PD with subsequent early initiation of PD treatment. However a further positive effect of LD may be the growth hormone inducing and thus putative regenerative effects of LD [1]. Growth hormone is under debate as an antiageing compound with multiple efficient regenerative modes of action in the human body [2]. However the ELLDOPA trial lasted 9 months; therefore it could not discuss additional aspects of LD treatment on progression of PD. These points are related to the inhibition of the main LD metabolising enzymes, catechol-O-methyltransferase (COMT), and dopa decarboxylase. Peripheral dopa decarboxylase inhibition (DDI) supports the predominant conversion of LD to its metabolite 3-O-methyldopa (3-OMD) by the ubiquitous enzyme COMT in blood, peripheral tissues and in nigrostriatal neurons (Figure 1). COMT has a broad detoxification potential in humans [3, 4]. In order to prolong the half life and thus to increase brain delivery of LD, COMT-inhibitors were developed as adjuncts to LD/DDI application. Two compounds are currently available, the only peripherally acting entacapone and the additional centrally working tolcapone [5, 6]. COMT inhibition is also under suspicion to prevent motor complications since the rise and fall of LD thus striatal dopamine are less intense during COMT inhibition, that is, entacapone. This supports the concept of continuous dopaminergic stimulation due to a more continuous LD brain delivery [7]. But COMT inhibition has
This is a transsulfuration process [20, 21]. The homocysteine derivative, cysteine, is a sulphydryl-containing amino acid with structural and chemical properties similar to those of homocysteine. Oxidation of cysteine in vitro also promotes several processes considered to be involved in atherogenesis and thrombogenesis. Cysteine has a cytotoxic effect in vitro against several cell types [20]. Cysteine forms an adduct with nitric oxide and may thereby impair endothelial function. Nitric oxide is believed to play a crucial role in the pathogenesis of chronic neurodegenerative processes [20]. High levels of homocysteine cause complex changes in cysteine levels and the overall aminothiol status in plasma [22]. A number of trials showed a close relationship between homocysteine and cysteine metabolism in various disease entities [20]. In plasma, the cysteine concentration is 20-fold higher than the homocysteine level. Rare information is available on cysteine levels in PD patients. Previous trials reported no relevant alterations of cysteine concentrations in PD patients, but moment of blood sampling in relation to intake of LD was not defined in detail in that studies [23–26]. A more recent study defined the moment of blood sampling one hour after LD/DCI intake and divided the cohort of PD patients according to their homocysteine level above the threshold of 15 µmol/L [27]. Only PD patients with an elevation of plasmatic homocysteine above 15 also showed an increase of cysteine plasma levels. This could have been due to the significant higher dosing of daily LD/DCI and the significant higher morning LD/DCI intake of PD patients with a homocysteine concentration above 15 in comparison with the remaining PD patients and the controls. The found significant correlations between morning LD/DCI dosages and cysteine, respectively, homocysteine concentrations, supported this view. Higher LD/DCI intake even seemed to influence cysteinyl-glycine levels due to the significant correlations in patients with elevated homocysteine concentrations [27].

### 2. Homocysteine and Arteriosclerosis

Both prospective and case-control studies have shown that an elevated plasma total homocysteine level is an independent risk factor for occlusive vascular disease. Various mechanisms have been suggested for the vascular lesions associated with hyperhomocysteinemia. The redox property of the sulphydryl group of homocysteine, leading to the formation of reactive oxygen species, is believed to play a pivotal role. This supports substantial impairment of endothelial function and subsequent atherosclerosis [17, 18]. Tie consequence is a vicious circle, since arteriosclerotic disease of striatal cerebral vessels hypothetically results in subsequent onset of increased susceptibility to impaired mitochondrial energy metabolism, oxidative stress, and basal ganglia circuit dysfunction, all of which represent typical, pathophysiologic features of PD [19].

### 3. Cysteine and Arteriosclerosis

Homocysteine is removed either by methylation to methionine or by its irreversible conversion to cysteine (Figure 1). Further beneficial effects on the LD-associated homocysteine increase [8]. Long-term LD intake promotes homocysteine elevation, which has an atherosclerosis promoting effect. An occurrence of increased hazard ratios for both ischemic heart and cerebrovascular disease is known in LD/DDI-treated PD patients, who were predominantly treated with LD monotherapy during the LD era [9, 10]. The conversion of LD to 3-OMD via the COMT requires Mg²⁺ as cofactor and S-adenosylmethionine as methyl donor [11]. Thus O-methylation of LD to 3-OMD is associated with conversion of S-adenosylmethionine to S-adenosylhomocysteine and subsequently homocysteine. Elevated homocysteine levels appeared in treated PD patients compared to matched controls [12, 13]. Treated but not previously untreated PD patients showed augmented homocysteine plasma concentrations [14, 15]. The relationship between homocysteine and 3-OMD plasma levels provided further evidence for an impact of LD on homocysteine [16].

### 4. Cysteinyl-Glycine and Arteriosclerosis

Metabolically interrelated to homocysteine and cysteine is cysteinyl-glycine. In plasma, this thiol species also determines the redox milieu and free radical generation rates. In another neurodegenerative disorder, Alzheimer’s disease, cysteinyl-glycine was reduced in plasma. Cysteinyl-glycine is a metabolite of glutathione. Glutathione depletion plays a crucial role in chronic neurodegeneration, since this leads to the accumulation of reactive oxygen species and, ultimately, oxidative stress related, apoptotic cell death, both of which are looked upon as essential pathophysiologic features of PD [22, 28]. All these thiol species—homocysteine, cysteine, and cysteinyl-glycine—exist in plasma in reduced, oxidized, and protein-bound forms, interacting with each other through redox and disulfide exchange reactions. Thus, hyperhomocysteinemia should not be considered as an isolated factor in relation to vascular disease and chronic neurodegeneration. The associated changes in other plasma aminothiols may modulate or even mediate atherogenesis, thrombogenesis, and the neurodegenerative process [20].
5. LD-Associated Homocysteine Increase and Clinical Consequences

Homocysteine increase may predispose for neuronal dysfunction and brain atrophy [29, 30]. This toxic impact of homocysteine on neuronal cell death was shown in sural nerves of PD patients and in animal trials [19, 31]. Thus, long-term LD administration may be indirect toxic to neuronal function due to homocysteine elevation [19, 26, 32]. Increase of vascular disease-related thickening of the intima media complex and an elevated risk for onset of neuropsychiatric complications, like depression or deteriorated cognitive function, were also described in association with augmented homocysteine levels in LD/DDI-treated PD patients [33–36]. However, the outcomes of more recent trials were only partial confirmatory [37, 38]. This could have been due to a relative low number of participants, design, and duration of these studies. Moreover, variations of the distribution of the polymorphism of the Methylene-tetrahydrofolate reductase (MTHFR) gene could have differed within the various investigated cohorts. In particular, the homozygote MTHFR T/T allele promotes elevated homocysteine levels [33, 39, 40]. Further causes for the controversial outcomes could result from variations of plasma bioavailability of LD. This depends on the gastrointestinal absorption and transport via the gastrointestinal amino acid transporter system. Both of them are influenced by gastric emptying time, which, if reduced, could decrease plasma appearance of LD [41]. Absorption of LD improves with progression of PD and chronic LD intake. As a result, this may support the risk of a LD/DDI-associated homocysteine increase [42, 43]. A further influence on LD metabolism may result from body weight. It is known that low body weight is associated with higher plasma LD levels [44]. All these components, related with LD metabolism, could also have influenced outcomes of trials on the clinical long-term consequences of the LD/DDI-induced homocysteine increase in PD patients. Homocysteine levels were mostly determined one time only in those investigations. A more recent study describes plasma metabolism of LD as essentially contributing component for homocysteine elevation after one time LD/DDI administration [45].

6. Homocysteine Increase and LD Degradation

LD metabolism is mainly influenced by the enzyme activities of dopadecarboxylase and COMT; the efficacy of inhibitors of these enzymes might also impact homocysteine levels. In this respect, application of LD only with a DDI supports homocysteine elevation, whereas LD administration with inhibitors of the both LD metabolizing enzymes may reduce the risk of homocysteine elevation [39, 46]. Experimental animal studies with a one single LD dose acute treatment paradigm also showed an impact on homocysteine generation even after 120 minutes [47–49]. Combination of COMT-inhibitors with LD/DDI also decreases homocysteine generation as shown with tolcapone [46]. In rats, this was also demonstrated with the COMT inhibitor entacapone, but this is under debate [47]. Observational European nonprospective investigations reported lower homocysteine levels in entacapone-treated patients [50–52]. A prospective clinical study on the effect of entacapone application on homocysteine levels was negative in PD patients. This trial might have been under powered because of the folic acid supplementation in the American and Canadian diet, leading to a milder homocysteine increase than expected [53, 54]. Currently these two COMT inhibitors are available, but their modes of action essentially differ. Tolcapone also acts in the brain, whereas entacapone only operates in the periphery [5, 6, 55]. This pharmacological difference between both compounds warrants a more general discussion; whether peripheral or additional central COMT-inhibition is more suitable to modulate homocysteine synthesis in the long term. Experimental investigations in cell culture models showed that COMT activation caused a sustained homocysteine generation in astrocytes and transport to neurons [26, 56]. Central COMT inhibition may even reduce homocysteine levels in glial and neuronal cells and therefore neurotoxic effects of homocysteine. But exclusive central COMT inhibition may also support central dopamine metabolism via monoaminooxidase (MAO), which generates neuronal oxidative stress [57]. Consequently, one may suggest to perform central COMT inhibition only with central MAO-B blocking to reduce free radical synthesis [3]. However, this combination may support glial dopamine metabolism via MAO-A and reduce the glial oxidation capacity of N-methylated tetrahydroisoquinolines (TIQ) to their neuronal neurotoxic cations [58]. But central COMT inhibition may also decrease O-methylation of endogenous TIQ to 1-Methyl-TIQ with their neuronal free radical synthesis reducing properties [58]. Therefore this shift from O-methylation to N-methylation as a consequence of central COMT inhibition may further enhance synthesis N-methylated TIQ, which induces PD [58, 59].

7. LD Associated-Homocysteine Increase and Vitamine Supplementation

Vitamine B complex and folic acid reduce homocysteine, since in example, folic acid catalyses metabolism of homocysteine to methionine [60]. Folic acid intake, which promotes the cobalamin dependent degradation of homocysteine to methionine, or supplementation of B6, which supports irreversible metabolism of homocysteine to cysteine, may help [21, 40]. But one animal study showed that folic acid coadministration did not prevent LD-associated hyperhomocysteinemia [48].

8. Conclusion and Hypothesis

LD is best available compound for PD treatment. Its delivery to the brain should be more continuous to avoid onset of motor complications. Long term LD application increases homocysteine levels in PD patients. This may contribute to onset of psychiatric side effects, neuronal degeneration, and vascular disease in PD patients. Treatment options
for the LD-mediated homocysteine toxicity is vitamin supplementation, that is, folic acid and LD application with COMT inhibition. The possible increased risk for synthesis of free radicals and N-methylated TIQ in association with central COMT inhibition may suggest peripheral COMT inhibition as safer adjunct for LD/DDI therapy.

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