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MOVEMENT DISORDERS - SHORT COMMUNICATION

Elevation of total homocysteine levels in patients with Parkinson's disease treated with duodenal levodopa/carbidopa gel

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Abstract Levodopa/carbidopa (LD/CD) application elevates total plasma homocysteine (thcys). We determined thcys-, LD- and 3-*O*-methyldopa (3-OMD) concentrations in 28 patients with Parkinson's disease (PD) on a LD/CD duodenal gel treatment. We found a distinct thcys increase (29.52 ± 28.98 µmol/l [median ± SD]) above the 15 µmol/l threshold and a significant ($R = 0.7$) correlation between LD and 3-OMD. thcys ascent was observed in relation with the onset of atherosclerosis, non-motor symptoms and polyneuropathy in PD patients in the long term.

Keywords Duodenal levodopa/carbidopa infusion · Homocysteine · Parkinson's disease

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Introduction

Chronic and acute, repeated intake of levodopa (LD) elevates total levels of homocysteine (thcys) with its half life of approximately 24 h in plasma (Hortin et al. 2006; Müller 2008; Müller and Kuhn 2009; Müller and Muhlack 2010). Therefore, thcys concentrations accumulate in association with application of high or well absorbed, acute and chronic intake of LD/dopa decarboxylase inhibitor (DDI) formulations (Müller et al. 2004, Müller and Kuhn 2009). This thcys ascent is under suspicion to contribute to the onset of atherosclerotic and neuropsychiatric symptoms and to accelerate an ensuing central nigrostriatal neurodegeneration in PD patients according to clinical and experimental trials (Rogers et al. 2003; O'Suilleabhain et al. 2004; Müller et al. 2004; Lee et al. 2005; Chandra et al. 2006; Imamura et al. 2007). In this respect, monitoring of thcys may also serve as biomarker for the detoxification potential of endogenous, exogenous and environmental toxins. These substrates may also accumulate in the peripheral and central nervous system, as homocysteine synthesis is associated with *O*-methylation, which has a broad detoxification potential (Müller 2010). Neurotoxins cause axonal polyneuropathy. Clinical and neurophysiological signs of neuronal degeneration occurred in sural nerves of PD patients in relation to elevated thcy levels during a chronic oral LD/dopa decarboxylase inhibitor (DDI) treatment regimen (Müller et al. 2004). Case reports described reversible polyneuropathy and mental retardation during duodenal LD/carbidopa (CD) gel application with an up to fourfold thcys ascent above the 15 µmol/l threshold (Hortin et al. 2006; Onofrj et al. 2009; Manca et al. 2009). Generally, duodenal LD/CD infusion improves motor complications in PD patients according to the concept of continuous dopaminergic stimulation, but mostly

PD patients receive several grams of LD/CD in monotherapy (Nyholm et al. 2005; Meiler et al. 2008). Therefore, determination of thcys values makes sense, since thcys increase is discussed as a risk factor for onset of atherosclerosis related disorders and non-motor symptoms of PD in the long term (O'Suilleabhain et al. 2004). Objectives were to investigate the acute effects of LD/CD gel administration on L-dopa-, 3-OMD-, and thcys concentrations in PD patients.

Subjects

The 28 participating PD patients (age 69.54 ± 5.59 years [mean ± SD]); Hoehn and Yahr stage 3.75 ± 0.67; Unified Parkinson's Disease Rating Scale (UPDRS) mental behavior 4.46 ± 2.82; UPDRS activities of daily living 21 ± 5.77; UPDRS motor examination 30.85 ± 5.93; UPDRS motor complications 5.62 ± 2.36; men 20; women 8; duration of disease 15.69 ± 5.41 years; LD/CD duodenal gel infusion mode [morning dose: 7.65 ± 4.83 ml, continuous flow rate 4.45 ± 2.03 ml/h, extra dose 2.54 ± 1.14 ml) fulfilled the clinical diagnostic UK Brain bank criteria for PD. They received LD/CD duodenal gel infusions in a chronic fashion over an interval of at least 4 months. Six patients additionally took compounds, which interfere with homocysteine generation (4 tolcapone, 1 folic acid, 1 B_6/B_{12} formulation) (Müller and Kuhn 2006).

Design

Blood samples were taken after at least 2 h of exposure to the LD/CD duodenal gel administration before lunch. Thus, we considered the acute impact of LD/DDI application on thcys levels. Blood specimen were at once centrifuged, then decanted and stored at −80°C for determinations of L-dopa, 3-OMD and thcys. The period between freezing and work up of the plasma samples was no longer than 3 months. Reversed-phase high-performance liquid chromatography (HPLC) was employed in combination with electrochemical detection for the measurement of L-dopa/3-OMD levels in plasma, which was diluted with a factor of 1:1.95 before assessment. thcys was measured by an automated HPLC with reverse-phase separation and fluorescent detection, by $NaBH_4$/mBrB reduction followed by monobromobimane derivatization.

Statistics

ANOVA with the Tukey's Honest significant test (Spojtvolle/Stolline) was employed for comparisons. Linear regression was used for the correlation analysis.

Ethics

All participants gave written informed consent. The study was approved by the local ethics committee of the Ruhr University of Bochum.

Results

Figure 1 shows the thcys values of all PD patients observed during ongoing duodenal LD/CD infusion. No patient was below the 15 µmol/l threshold. There were no associations between all the assessed parameters and the data of the application modes. This graph illustrates that additional tolcapone- and/or vitamin B_6-, B_{12}-, folic acid intake does not lower thcys below 15 µmol/l. Figure 2a exemplifies the ($p < 0.001$) correlation between LD- and 3-OMD plasma concentrations ($R = 0.7$). Figure 2b describes no significant differences to ascending 3-OMD plasma levels following standardised triple intake of an oral 100 mg LD/CD formulation after 240 min and later. Data were taken from (Müller et al. 2006).

Discussion

We show a distinct elevation of thcys in PD patients during ongoing duodenal LD/CD gel infusion. We assume that no further thcys decrease will occur in our cohort of long-term duodenal LD/CD gel-treated PD patients. Similar to thcys, the plasma half life of 3-OMD is distinct longer when compared with the one of LD (Benetello et al. 1997; Antonini et al. 2010). Therefore, one may hypothesize that 3-OMD accumulates over time during chronic duodenal LD/CD gel infusion. However, we confirm that 3-OMD does not enhance and is similar to ranges observed after triple application of 100 mg LD/CD (Fig. 2b) (Antonini et al. 2010). Thus, we assume that a certain balance between synthesis and degradation of 3-OMD is developed during repeated duodenal LD/CD gel infusion. The correlation between LD- and 3-OMD plasma concentrations supports this hypothesis (Fig. 2a, b). An essential precondition for this equilibrium between LD- and 3-OMD bioavailability is that LD is predominantly metabolised to 3-OMD in the presence of a DDI, like CD, by catechol-*O*-methyltransferase (COMT). COMT is the essential enzyme for this *O*-methylation of LD, which demands for a methyl group transfer from the donor *S*-adenosylmethionine (SAM). As one consequence, SAM is transformed into the short living *S*-adenosyl-homocysteine and then to homocysteine. Homocysteine is degraded either by its irreversible conversion to cysteine or by reversible re-methylation to methionine, the metabolic precursor of SAM. There are two

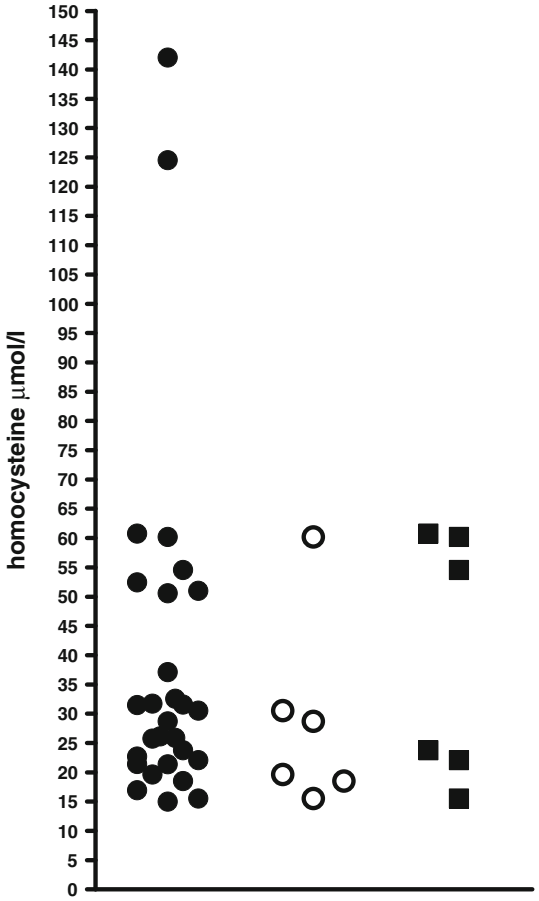

Fig. 1 Plasma concentrations of total homocysteine in PD patients on ongoing duodenal LD/CD gel infusion therapy. *Filled circles* values of all participants, *open circles* levels of PD patients with 24 h lasting LD/CD gel administration, *filled squares* concentration of PD patients, who additionally took tolcapone, respectively, vitamins such as folic acid, B_6 or B_{12}

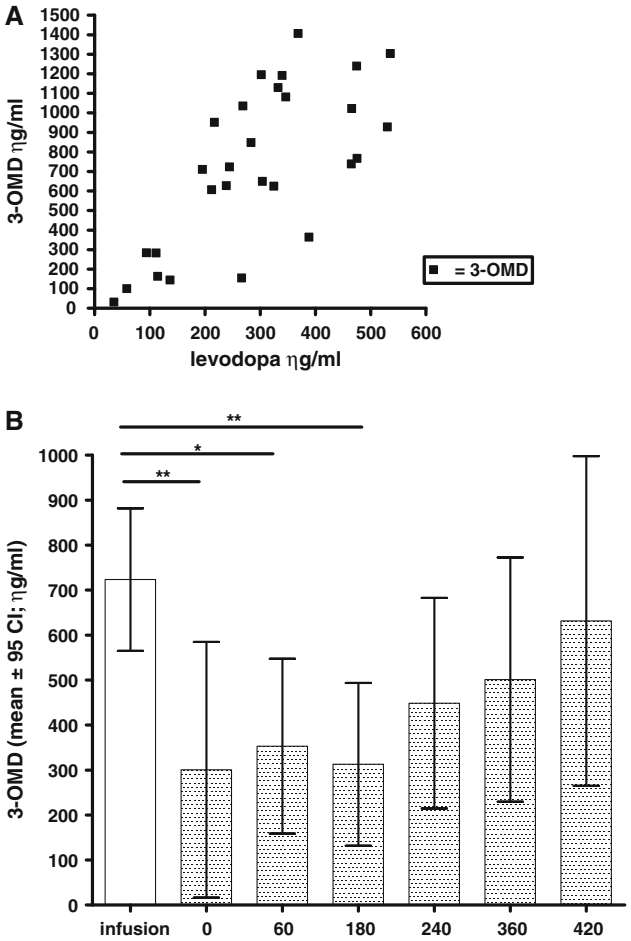

Fig. 2 **a** Correlation between levodopa and 3-*O*-methyldopa plasma concentrations. **b** Significant ($F = 3.74$; $p = 0.002$) comparison between 3-*O*-methyldopa plasma concentrations during duodenal LD/CD infusion therapy and repeated LD/CD application. = parameters during ongoing LD/CD gel infusion; ▒ values under oral therapy with three LD/CD intakes; 0 = baseline, 60, 120, 180, 240, 360, 420 = min following first LD/CD intake, data taken from Müller et al. (2006); $^{**}p < 0.001$; $^{*}p < 0.05$

separate re-methylation pathways, catalyzed by betaine: homocysteine methyltransferase and methionine synthase. These reactions that remove homocysteine are very sensitive to vitamin B status. The transsulfuration enzymes contain pyridoxal phosphate, while methionine synthase contains cobalamin and receives its methyl group from the folic acid one-carbon pool. Generally, vitamin supplementation and other non-genetic factors may amplify or mask phenotypic expression of genetic polymorphisms or genetic defects, both of which impact the evaluation of hyperhomocysteinaemia. These genetic polymorphisms or defects are in example heterozygous cystathionine synthase deficiency or certain thermolabile 5,10-methylenetetrahydrofolate reductase (MTFHR) variants.

A certain thcys decrease over weeks was described in the context of duodenal LD/CD application in PD patients (Antonini et al., 2010). But this trial also reports distinct higher thcys levels with values between approximately 40 and 120 µmol/l in their 19 PD patients on a duodenal LD/CD gel regimen (Antonini et al. 2010). Nevertheless, we assume that onset of axonal degeneration during L-dopa infusion is not due to the application of L-dopa itself. This serious side effect may occur as consequence of secondary metabolic changes such as homocysteine elevation and associated decay of vitamins like folic acid, B_6 or B_{12} (Manca et al. 2009; Antonini et al. 2010). Accordingly, case series on the origin of the polyneuropathy in chronic L-dopa/dopa decarboxylase inhibitor treated PD patients reported low B_{12} levels and elevated homocysteine concentrations. They recommend intramuscular B_{12}- and oral folate supplementation, as they then observed improvement of polyneuropathy symptoms in their PD patients. However, they stress that one should advocate this only for patients with confirmed methylmomalonic acid/homocysteine elevations (Toth et al. 2008, 2010a, b).

As homocysteine synthesis represents a secondary reaction product of *O*-methylation of L-dopa to 3-OMD, one may hypothesize that COMT inhibitors and/or vitamin supplementation may exert a certain preventive effect on the onset of axonal polyneuropathy during duodenal LD/CD administration. However, our present values also show elevated thcys levels with concomitant tolcapone- and/or vitamin intake (Fig. 1) (Müller et al. 2006). From this point of view, one must conclude that additional concomitant COMT inhibition and/or vitamin supplementation may only provide a limited impact on thcys increase.

Investigations in dopamine synthesizing PC12 cell cultures demonstrated that a weakened NGF synthesis occurs with an increase in homocysteine (Zhao et al. 2002). Therefore, we suggest that a hypothetical NGF deprivation is additionally responsible for occurrence of polyneuropathy in some PD patients under LD/CD infusion therapy. In contrast, the dopa decarboxylase-dependent LD metabolite dopamine may counteract this NGF deprivation (Furukawa et al. 1989; Müller et al. 2005).

Limitations of our investigation are that we did not investigate the MTHFR status, vitamin levels, nerve growth factor parameters and the presence of clinical signs of peripheral neuronal axonal damage in our cohort due to technical reasons and the relative low number of study participants.

In conclusion, we suggest serial measurement of thcy levels and application of all preventive and therapeutic options to lower thcys concentrations in duodenal LD/CD gel-treated PD patients.

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