The effectiveness of choline citrate infusions monitored by lymphocyte transformation test (LTT) in multiple sclerosis. A new approach to the diagnosis and treatment of the disease

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Abstract

The efficacy of intravenous choline citrate infusions was investigated in 34 patients with multiple sclerosis (MS) by clinical evaluation and by monitoring of lymphocyte proliferation in vitro against fragments of myelin basic protein (MOG-35-55, MBP15-31, PLP 39-15) over a period of 12 weeks. Patients have been diagnosed with MS at least one year before entering the study and suffered from mild relapsing/remitting course to long-term chronic progressive disease.

Twenty one patients exhibited positive lymphocyte proliferation to myelin fragments prior to treatment and were therefore selected for further studies. Choline citrate was administered with a dosage of 1200mg/ 2× week for a period of 3 months. This treatment resulted in a significant decrease of lymphocyte proliferation to neural fragments (MOG- 35-55, MBP15-31) in lymphocyte transformation test (LTT). There was no significant SI change of PLP Peptide (PLP 39-15) LTT found after treatment with choline citrate. During the 3 mo observation period, patients remained stable and no side-effects of the treatment were observed. In addition, some patients reported long-lasting improvement (less paresthesia and increase of muscle strength in lower extremities) which was demonstrated up to 3 years later. In one spectacular case a commercial pilot was able to return to duty again after treatment. This pilot was allowed back in to his position as a commercial flying cockpit member and is on duty for more than 4 yrs now.

INTRODUCTION

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) and its pathology is characterized by perivascular mononuclear cell infiltrates preceding myelin loss. The cause of MS is yet unknown, but it has been postulated that the myelin damage is immune-mediated, either secondary to a viral infection or due a autoimmune process. Furthermore there is evidence that heavy metal burden has an impact on immune dysregulation related to MS (Yaqob *et al* 2006). MS Patients have been shown to have T cells reactive to a number of myelin antigens (Lovett-Racke *et al* 1998; Prochazkova *et al* 2004).

CLINICAL STUDY

There is definite in vitro evidence of difference in the activation state of myelin-reactive T-cells in the central nervous system of patients with MS. It has been suggested that autoreactive T cells have a pathogenic role in the disease (Zhang *et al* 1994).

The inoculation of MOG peptides into C57BL/6 mice induced CD4 (+) and CD8(+) T cells, and adoptive transfer of in vitro activated lymphocytes induced experimental allergic encephalomyelitis (EAE). EAE is considered to be an animal model for MS in naïve recipient mice (Weber *et al* 1994).

Recent experiments have also indicated that autoreactive T cells are not deleted in the thymus and circulate in the periphery. Also myelin specific T cells were not detectable in the CSF samples of patients suffering from other neurological disease than MS showing them to be a specific marker (Chou *et al* 1992). Myelin basic protein specific cells however showed functional heterogeneity with regards to their cytotoxic activity against human astrocytes and monocytes (Weber *et al* 1994). A study with multiple human MBP preparations found that at least 67% of MS Patients were sensitized more significantly to human MBP compared with 27% of normal. These results indicate that different stages of MS can be monitored by sphingolipid derived MOG and MBP in LTT (Chou *et al* 1992).

We treated our patients with an off labeled drug choline citrate because literature indicated to us that choline chlorid is involved in myelin chemistry and the regulation of inflammation in CNS. This was concluded from MR Studies on MS patients showing a peak in MR spectra associated with membrane phospholipids even before the appearance of MRI-visible MS lesions. The results of this study were consistent with focal pre-lesional myelin membrane pathology at least 12 months before lesions become visible on conventional MRI (Tartaglia *et al* 2002). Others found an increase in choline, betaine, and phosphorylcholine (PC) as well as a reduction in N-acetylaspartate (NAA), aspartate, N-acetylaspartatylglutamate and inositol in vitro in MS patients. They conclude that the increase of choline was due to inflammation (Brenner *et al* 1993).

Recent experiments have further indicated that autoreactive T cells are not deleted in the thymus but do circulate in the peripheral blood. Since myelin basis protein (MBP)- specific T cells are detectable in the cerebrospinal fluid and in the blood samples of MS patients but not in patients suffering from other neurological diseases, they are considered to be a specific marker for MS (Chou *et al* 1992). A study with several human MBP preparations found that at least 67% of MS patients were sensitized more significantly to human MBP as compared to 27% of normal subjects. We used the lymphocyte transformation test (LTT) to monitor the efficacy of treatment with an off labeled drug- choline citrate- given intravenously for twice a week for 3 months.

METHODS AND PATIENTS

A cohort of 34 Patients diagnosed MS were monitored for MOG-35-55, MBP15-31 and PLP 39-15 in Lymphocyte-Transformation Test (LTT). Patients had been diagnosed MS at least one year before entering the study ranging from a mild relapsing/remitting course to long term chronic progressive disease. The age range of the patients was 25-46 year with a mean age of 38yr.

Infusions with choline citrate were based on ampoules with choline citrate 300,2 mg each as the effective substance (Neurotropan® Fa. Phönix Germany). Other compounds: hydrochloric acid, water for injection. We administered 4 ampoules in 250 ml physiological solution of NaCl fluid/2x week. I.t. Patients received a total dosage of 28,8g choline citrate in this treatment over a period of 3 months (12 weeks).

Laboratory investigations included complete blood count with differential and platelet count as well as serum values of liver enzymes, bilirubin, albumin, glucose creatine and urinary analysis in order to excude patients with other disease. Patients were also checked for ANA and peripheral blood cells. None of the patients had received steroids or other immunosuppression for at least 12 month before administering choline infusions. The LTT was carried out with 10 % heat-inactivated pooled human AB Serum. Subsequently, 20 µl recombinant human interferon- alpha 2a (Biosource, Giessen, Germany) stock solution prediluted in RPMI to 1250 IU/ml, was added to the plated cells immediately before adding the appropriate antigen. The resulting final concentration of IFN-alpha in the culture was 125 IU/ml. Differences in the stimulation indices between the LTT records before and after treatment were evaluated for significance using the Mann-Whitney U-Test. Results were considered statistically significant at $P < 0,05$.

RESULTS

Of 34 non randomized patients N= 21 (62 %) had positive stimulation indices (SI = LTT stimulation Index > 2) in their essay and therefore where selected for treatment with choline citrate i.v. In these 21 cases MBP Peptide 15-31 as well as MOG Peptide 35-55 LTT stimulation indices where significantly lower after treatment with choline citrate (p=0,027; p=0,016) in LTT in comparison to LTT SI prior to treatment. There was no significant change of PLP Peptide 13-91 stimulation indices after treatment with choline citrate (p=0,102) (p= Wilcoxon-Test exact significance (2-sided)).

DISCUSSION

Our study was designed to prove a cinical impact of i.v. choline citrate (1200mg/2× week i.v.) by means of MOG, MBP and PLP LTT in a cohort of diagnosed MS patients. However, we have to admit that our study setting was not able to provide final conclusions on the

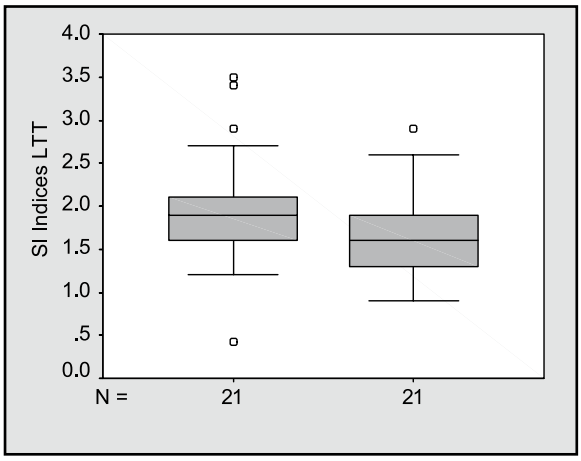

Figure 1: MBP 15-31 LTT stimulation indices prior and after treatment with choline citrate.

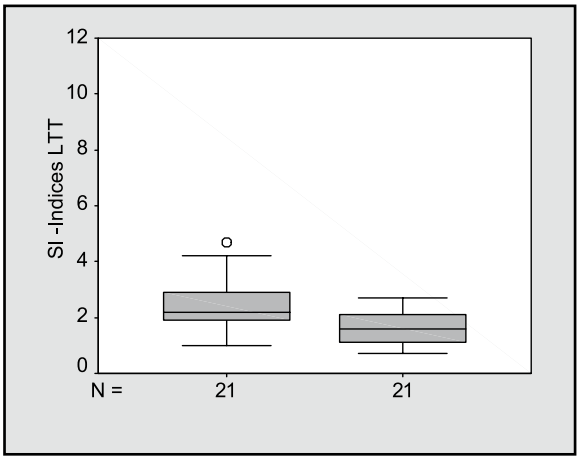

Figure 2: MOG 35-55 LTT stimulation indices prior and after treatment with choline citrate.

effectiveness of i.v.-choline citrate treatment in MS. The number and included patients was to small to achieve fully representative data. However, in concordance with recent research data we are able to resume an anti-inflammatory effect of this therapeutic regime from our results.

It is not to our knowledge that the effectiveness of treatment in MS has ever been followed up by LTT-protocols. For this reason we are not able to compare our results with prior studies. Presenting our results we even have to admit that the percentage of primed myelin-reactive T cells in MS patients has been discussed in dependence of the extent of the blood-brain barrier breakdown. This process was not followed up in our study. We suggest to include more laboratory test such as measuring Neuron specific enolase (NSE) in the blood of patients in further studies for this reason (Steinberg *et al* 1983). In addition it has been shown that the population of MBP-reactive T cells appears to be changing during the course of disease which was related to the amount of recruited and activated T cells (Lovett-Racke *et al* 1997). The consistency of the LTT protocols will therefore need to go under further evaluation.

Literature studies indicate that choline citrate is involved in myelin chemistry and down regulates inflammation in CNS (Chou *et al* 1992). Choline citrate has been described as a substance that increases ATP and perfusion in CNS and regulates lymphocyte activity (Maiwald 2001). Others found an increase in choline, betaine, and phosphorylcholine (PC) as well as a reduction in N-acetylaspartate (NAA), aspartate, N-acetylaspartatylglutamate and inositol in vitro in MS patients. They conclude that the increase of choline was due to inflammation (Brenner *et al* 1993).

Besides these facts, we were in favor of the LTT to study the effects of choline citrate infusions because previous research had proven stimulation indices of LTT (MOG and MBP) in positive correlation with other markers of CNS inflammation (Weber *et al* 1994).

In our study positive MS patients received choline citrate infusions (1200mg i.v.) over a period of 12 weeks twice a week. This treatment contributed to a significant decrease of LTT-levels with regards to MOG and MBP. However, there was no significant change of PLP Peptide 13-91 stimulation indices found after treatment with choline citrate. This decrease may be related to the anti-inflammatory effect of choline citrate in CNS.

Comparisons of relative metabolite levels between MS patients and controls with regard to gray matter metabolic abnormalities and white matter inflammation showed significant decrease of N-acetyl aspartate (NAA) levels and an increase in choline-containing compounds (Cho) levels (Van Au Duong *et al* 2007). Choline storage in CNS may therefore possibly be understood as an physiological inflammatory response attempting to down regulate inflammation. The systemic anti- inflammatory effectiveness of choline treatment has been proved in other studies. Choline treatment in sensitized mice significantly inhibited eosinophilic airway inflammation and EPO activity. It also reduced IgE and IgG1 production and inhibited the release of Th2 cytokines and leukotrienes (Mehta *et al* 2007). A Further study demonstrated that dietary gangliosides inhibited pro-inflammatory signals in the intestine and blood and suggested the therapeutic potential in the treatment and management of acute local and systemic inflammatory diseases (Park *et al* 2007).

Our results were taken under clinical conditions treating MS diagnosed patients with different symptoms and varying courses of the disease. We where not able to follow up clinical reports on our patients for a longer period (more than over three month) of time to prove a long term lasting effectiveness of our treatment. However none of our patients showed any neurological symptoms of severe deterioration under the treatment nor were any side effects recognized.

A special remarkable success was seen in a 33 yr old licensed air pilot. Prior this patient had received cortisone treatment in advance a yr ago which failed

to improve any of his symptoms. He was involved in our treatment procedure and choline citrate infusions stabilized his clinical condition to a degree that all final compulsive clinical neurological exams and test proved him physically capable to return to duty again. This pilot was allowed back in to his position as a commercial flying cockpit member and is on duty for more than 3 yrs now.

As a conclusion, we encourage further studies on the effectiveness of choline citrate in MS patients. Maybe the use of even more sensitive Lab diagnostic parameters such as MELISA will then entail more significant data (Valentine-Thon & Schiwara 2003).

This study was performed in accordance with the rules of local ethical committee.

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