SHORT COMMUNICATION

Effects of Repeated Intrathecal Triamcinolone-Acetonide Application on Cerebrospinal Fluid Biomarkers of Axonal Damage and Glial Activity in Multiple Sclerosis Patients

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Abstract

Background and Objectives   Multiple sclerosis (MS) is the most common inflammatory disease of the central nervous system in young adults. Over time, the disease progresses and, with accumulating disability, symptoms such as spasticity may occur. Although several treatment options are available, some patients may not respond to first-line therapeutics. However, some of these patients may benefit from intrathecally administered triamcinolone-acetonide (TCA), a derivative of glucocorticosteroids (GCS). GCS may have neurotoxic effects, and cell apoptosis may occur. The aim of this study was to investigate the effects of TCA on biomarkers in the cerebrospinal fluid (CSF) suggestive of neurodegeneration.

Methods   In order to assess neurotoxic effects of TCA, neurofilament heavy-chain (NfH)^{SMI35}, tau protein, and S-100B protein levels were determined before and during treatment with TCA in 54 patients with primary progressive MS, as well as relapsing MS (relapsing–remitting and secondary progressive MS).

Results   NfH^{SMI35} levels in the CSF of patients treated with TCA intrathecally did not increase significantly during the treatment cycle ($p = 0.068$). After application of TCA, tau protein levels were increased significantly at day 4 ($p = 0.03$) and at day 8 ($p \leq 0.001$). S-100B protein levels decreased significantly ($p \leq 0.05$) during treatment with TCA.

Conclusion   NfH^{SMI35} levels did not change significantly; however, tau protein levels did increase significantly within the reference range. Taking these findings together, the long-term effects of TCA on NfH^{SMI35} and tau protein levels need to be investigated further to understand whether levels of both biomarkers will change over repeated TCA applications. Interestingly, S-100B protein levels decreased

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significantly during the first applications, which may have represented reduced astrocytic activity during TCA treatment.

Key Points

Short-term intrathecal triamcinolone-acetonide (TCA) treatment significantly influences levels of two proteins expressed by astroglia: S-100B and tau protein, suggesting reduction of astrocytic activation

In this study, short-term intrathecal TCA treatment did not change neurofilament heavy-chain levels, suggesting the absence of a major neuroprotective treatment effect

Future treatment trials may investigate whether hyperacute treatment with intrathecal TCA may reduce the inflammatory component of glia in the setting of severe transverse myelitis in multiple sclerosis and possibly also neuromyelitis optica spectrum disorders, and thus improve the clinical outcome

1 Introduction

Multiple sclerosis (MS) is the most common inflammatory disease of the central nervous system in young adults [1]. The estimated worldwide prevalence of MS is 2.1 million people [2]. Over time, the disease disability accumulates, and most patients may enter ongoing progression (progressive MS), whereas acute exacerbation may become less likely [3]. With accumulating disability, patients may suffer from spasms. Consequently, patients report impaired walking and may experience other symptoms, such as unpleasant sensations, pain, and incontinence [4]. Symptomatic treatment is essential for these symptoms. Besides impaired quality of life, spasticity represents a tremendous cost factor. A study in Sweden revealed that annual costs are 2.4 times higher in patients with severe spasticity than in those with only mild spasticity [5–7]. Spasmolytic therapeutics taken orally may have systemic side effects, and thus their usage is limited. Intrathecal baclofen therapy and baclofen pumps offer a way to deal with spasticity, but besides the cost factors, systemic side effects and infections are common [8].

Intrathecally applied glucocorticosteroids (GCS) have been used since the 1950s. As therapeutic agents, hydrocortisone and methylprednisolone acetonide have been used but failed to show immunomodulatory effects on the course of disease. Side effects such as urinary incontinence, constrictive arachnoiditis, aseptic meningitis, and

spinal cord lesions have been reported. However, positive effects on spasticity, with improvement of walking, have also been reported. A number of monocentric trials have shown positive effects of intrathecal GCS application on spasticity [9–12]. Intrathecal therapy with triamcinolone-acetonide (TCA), a derivative of GCS, has been shown to be safe, and no severe side effects have occurred [13]. Intrathecally applied GCS seems to have positive effects on spasticity and may spare MS patients the discomfort of wearing a corset.

Nevertheless, in vitro studies have shown that GCS may induce apoptosis in neuronal cells [14, 15]. This hypothesis is supported by observations in vivo [16]. It is unclear whether GCS or its additives may induce cell injury [17]. There is therefore a need to investigate the effects of intrathecal GCS on neurodegenerative markers in the cerebrospinal fluid (CSF) to address this issue.

Neurofilaments are a highly specific component of the axoskeleton and are released during axonal damage. Neurofilament heavy-chain (NfH) levels in body fluids are an established marker for estimation of axonal damage in a range of neurological disorders [18].

Tau protein, as a phosphorylated microtubule-associated protein, is known as a component of neuronal axons. Released into the CSF, it may serve as a biomarker of axonal damage, as shown by previous studies [19–21].

S-100B is a multigenic family of calcium-binding proteins found in astrocytes. Several studies have suggested that these proteins might play a certain role in the regulation of effector proteins and may influence specific parts of signaling pathways or cellular functions. S-100B proteins seem to possess both neuroprotective and neurotoxic properties, and have been used as a marker of astrocytic proliferation and gliosis [21–23].

The effects of TCA treatment are complex, and its pharmacodynamic effects on tau protein, S-100B, and NfH^{SMI35}, as potential predictors of axonal degeneration, glial activation, and astrogliosis in MS patients, have not been extensively characterized.

This prospective study aimed to investigate the effects of repeated TCA applications on the CSF markers suggestive of neurodegeneration: tau protein, S-100B, and NfH^{SMI35} in MS patients with severe spasticity.

2 Material and Methods

2.1 Patients

Fifty-four patients suffering from MS, according to the McDonald criteria [24], were consecutively recruited to participate in this multicentre study (see Table 1). All patients were clinically stable (i.e. they had experienced no

Table 1 Patient characteristics

Characteristic	All patients	Female patients	Male patients
Patients (*n*)	54	36	18
Age (years ± SD)	47.9 ± 9.7	48.7 ± 9.7	46.2 ± 9.9
Course of disease (*n*)			
Relapsing MS–remitting MS	46	33	13
Primary progressive MS	8	3	5
Age at onset of disease (years ± SD)	35.1 ± 8.7	35.5 ± 8.8	34.4 ± 8.8
Disease duration (years ± SD)	12.4 ± 8.4	13.0 ± 8.5	11.3 ± 8.1

MS multiple sclerosis, *SD* standard deviation

relapse or recent progression over the previous 3 months) and were not receiving other immunomodulating or immunosuppressive treatment. Two thirds of the included patients were female and were in the progressive stage of MS. Statistical analyses revealed a non-normal distribution, according to the Kolmogorov–Smirnov test (see Table 1).

All patients gave written and informed consent for participation in the trial, which was approved by the local ethics committee at the University of Rostock (Rostock, Germany) and registered with the German Clinical Trials Register (DRKS; registration no. DRKS00005671) [see http://www.drks.de and http://www.who.int/ictrp/en].

2.2 Procedures

Every second day, TCA was applied intrathecally. Depending on the spasticity, the dosage ranged from 40 mg to 80 mg. Furthermore, the applications were done three times or, in cases with severe spasticity, five times over the course of a week. TCA treatments were performed on days 0, 2, and 4 (in patients receiving three applications) and additionally on days 6 and 8 in those receiving five applications. In 26 patients, TCA was administered five times, whereas treatment was terminated after three applications in 28 patients because a sufficient antispastic effect had been achieved. The mean dosages over the cycle were 127.4 mg (standard deviation [SD] 22.3) in patients receiving three applications and 205.9 mg (SD 37.3) in those receiving five applications.

All lumbar punctures were performed as per routine clinical practice for therapeutic purposes only. Because of the invasiveness of the therapy, no CSF from control subjects was obtained. Sprotte® cannulas (Pajunk Medizintechnologie GmbH, Geisingen, Germany) were used for obtaining CSF. After each lumbar puncture, CSF was obtained in 10 ml polypropylene tubes and stored within 30 min in 1 ml aliquots at −70 °C.

2.3 Laboratory Analyses

The routine parameters of the CSF, including the cell count, total protein, albumin, lactate, quantitative immunoglobulin

(Ig)-G, and oligoclonal IgG bands (OCB), were quantified in the local laboratories at each centre. CSF protein biomarkers were quantified in dedicated laboratories (tau protein and S-100B in Ulm, Germany; NfH[SMI35] in London, UK). Cell-free CSF was shipped on dry ice and kept frozen on arrival without interruption of the cooling chain.

2.3.1 Measurement of Neurofilament (NfH[SMI35])

The original method has previously been described in detail [18]. In brief, immunoassays were performed for quantification of NfH. Mouse monoclonal anti-NfH antibody SMI35R was used for capture (Covance, Princeton, NJ, USA), rabbit polyclonal anti-NfH was used as the detecting antibody (Sigma-Aldrich, St. Louis, MO, USA), and horseradish-labeled swine polyclonal anti-rabbit antibody was used as the indicator antibody (Dako, Copenhagen, Denmark).

Chemicals were obtained from Sigma-Aldrich (ethylenediaminetetraacetic disodium salt [EDTA], sodium barbitone, sodium carbonate [Na_2CO_3], sodium hydrogen carbonate [$NaHCO_3$], Tween 20 [soap], bovine serum albumin [BSA]), from Merck (Darmstadt, Germany; hydrochloric acid [HCl]), and from Dako (tetramethylbenzidine [TMB]). For analysis, MaxiSorp microtiter plates (Life Technologies, Paisley, Scotland) were used.

2.3.2 Measurement of Tau Protein

Tau protein levels were determined using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) [InnoTest hTau; Innogenetics, Zwijndrecht, Belgium] based on the original method of Vandermeeren [25].

The assay was performed according to the protocol supplied with the kit, and CSF tau protein levels in the samples were estimated from standard curves made for each assay.

2.3.3 Measurement of S-100B Protein

S-100B protein levels were measured by means of an immunoluminometric assay kit (LIA-mat® Sangtec® 100;

AB Sangtec, Bromma, Sweden), which measures the b subunit of S-100B protein [26]. The assay was performed according to the manufacturer's protocol.

2.4 Statistical Analyses

Statistical analyses were performed with SPSS® version 15.0 (Chicago, IL, USA) and IBM® SPSS® Statistics version 20 software (Armonk, NY, USA). The Kolmogorov–Smirnov test was used for checking the distribution of all parameters. The collected data did not follow normal distribution. CSF parameters, socio-demographic parameters, and clinical parameters were analyzed with the Kruskal–Wallis one-way analysis of variance and the Mann–Whitney U test. For analysis over the course of time, the Friedman test and the Wilcoxon t test were used. Statistical significance was set at the 95 % confidence level ($p < 0.05$).

3 Results

Overall, the therapy was tolerated well. No infections were reported. Back pain and headache were rare. The low rate of headache may have been due to the fact that TCA suspension dissolved in saline was administered. The results did not differ significantly between the groups. The unbalanced design of the study may have limited the results. The clinical outcomes showed a significantly improved ($p \leq 0.001$) walking distance. On average, the

walking distance improved in all patients from 247 m (SD 302) to 322 m (SD 346). Spasticity was measured on the Ashworth Scale and improved significantly ($p \leq 0.001$) during TCA applications from 2.2 (SD 1.3) at baseline to 1.6 (SD 1.1) at the end of the cycle. Further analysis will be reported separately.

CSF markers were determined during application of TCA at baseline (the first application of TCA), on day 4 (the third application of TCA), and on day 8 (the fifth application of TCA). Patients were given TCA three times ($n = 54$), though in cases of severe spasticity ($n = 26$), TCA was applied five times. Besides the aforementioned markers of neurodegeneration, the cell count was determined as part of the routine assessment. Further CSF analysis revealed a decreasing cell count from 10.8 (SD 18.9) at baseline to 6.3 (SD 6.2) on day 8.

NfH^{SMI35} levels in the CSF of patients treated with TCA intrathecally did not increase significantly (baseline level: 0.11 ng/ml [SD 0.11]; level at third application [after two TCA applications]: 0.14 ng/ml [SD 0.15], $p = 0.053$; level at fifth application [after four TCA applications]: 0.16 ng/ml [SD 0.15], $p = 0.068$) during the treatment (see Fig. 1). In all patients, CSF NfH^{SMI35} levels remained within the normal range (maximum value 0.58 ng/ml, below the cut-off of 0.73 ng/ml) [15].

After application of TCA, tau protein levels were increased significantly on day 4 (from 194.07 pg/ml [SD 82.47] at baseline to 250.31 pg/ml [SD 168.84], $p = 0.03$) and on day 8 (245.54 pg/ml [SD 121.79], $p \leq 0.001$) [see Fig. 2].

Fig. 1 Concentration of NfH^{SMI35} during the first cycle of triamcinolone-acetonide (TCA) therapy. Statistical analysis: Friedman test, Wilcoxon test. NfH neurofilament heavy-chain
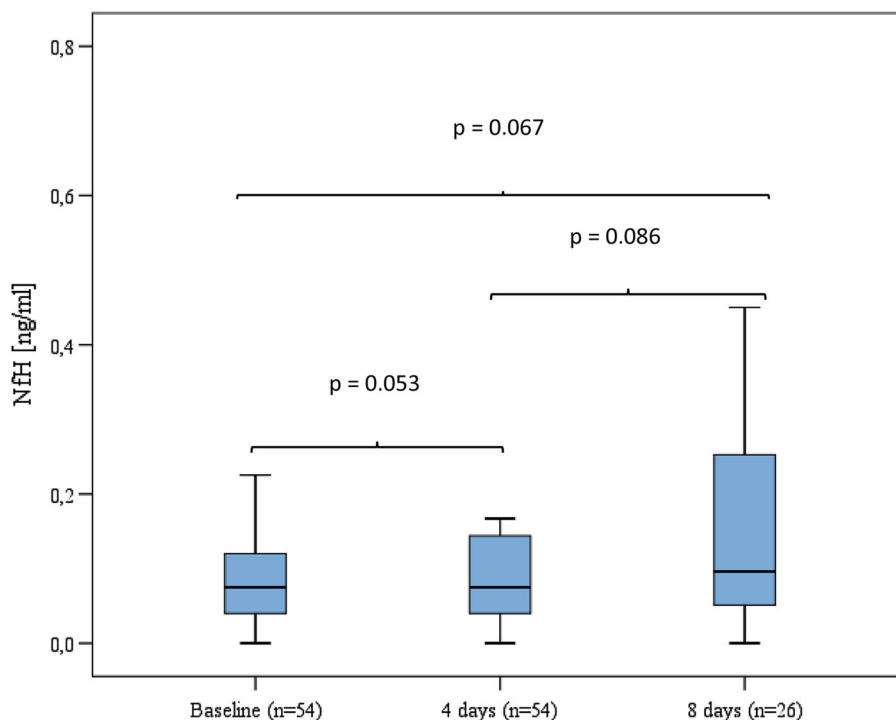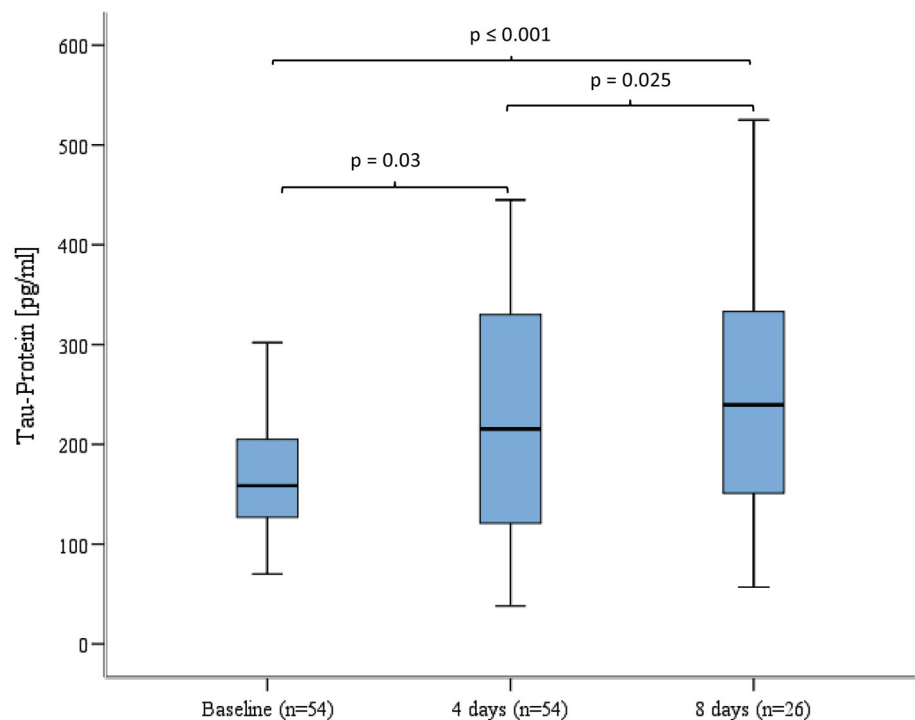
Fig. 2 Concentration of Tau protein during the first cycle of triamcinolone-acetonide (TCA) therapy. Statistical analysis: Friedman test, Wilcoxon test

S-100B protein levels decreased significantly over the complete cycle (from 1.18 mg/l [SD 0.66] at baseline to 1.00 mg/l [SD 0.44] on day 8, $p \leq 0.05$). The effect could already be seen by day 4 (1.01 [SD 0.46], $p \leq 0.05$) [see Fig. 3].

The results for NfH[SMI35], tau protein, and S-100B did not differ significantly between the relapsing MS–remitting MS patient group and the primary progressive MS patient group.

4 Discussion

Intrathecal TCA is an efficient therapeutic way to treat spasticity and spare MS patients the discomfort of wearing a corset. It has proven its efficacy in a number of trials [24–26]. In vitro experiments have suggested that GCS has neurotoxic properties [14, 15, 17].

In our trial, we measured the levels of NfH[SMI35], tau protein, and S-100B protein, which are well accepted as surrogate markers of axonal injury and degeneration [27], neuronal damage [20, 28], and activated astroglia, mainly reflecting parenchymal damage [22, 29].

NfH[SMI35], as a surrogate marker of axonal damage, was measured for the first time in patients receiving TCA treatment. The results did not reach significance and remained below the assumed critical value of 0.73 ng/ml. Neurofilament activity varies between the different stages of disease and is influenced by clinical activity. In respect thereof, all of our patients were stable with no relapse for

3 months prior to the first application of TCA and, although the patients were recruited at different stages of the disease (relapsing MS and progressive MS), with differing Expanded Disability Status Scale (EDSS) scores, the results with regard to NfH did not vary significantly in the intergroup comparison. This may have been due to the limited power of the study. Another limitation might be the fact that only patients with low NfH levels at baseline were included in the trial. It would have been interesting to see what would have happened to patients with pathological NfH levels at baseline.

The level of total tau protein significantly increased in comparison with baseline. However, the mean value did not reach the critical value of 300 pg/ml [28]. The question of to what extent such an increase has clinical implications remains open. In a study by Hoffmann et al. [10] in 27 patients, TCA was applied six times, and the levels of tau protein and S-100B did not increase significantly. Research into the long-term effects of TCA on tau protein levels may elucidate whether increased levels have further clinical implications. Currently, the importance of tau protein in MS patients is not completely understood. To what extent this increase could have been explained by release of tau protein from the cytoskeleton of neurons or from glial cells in our patients remains open. The importance of tau protein in neuroinflammation and neurodegeneration in MS is not fully understood. Whereas animal models have suggested a link between axonal damage and tau pathology [30], findings elucidating tau protein levels in the CSF of MS patients have been partly contradictory [31–33].
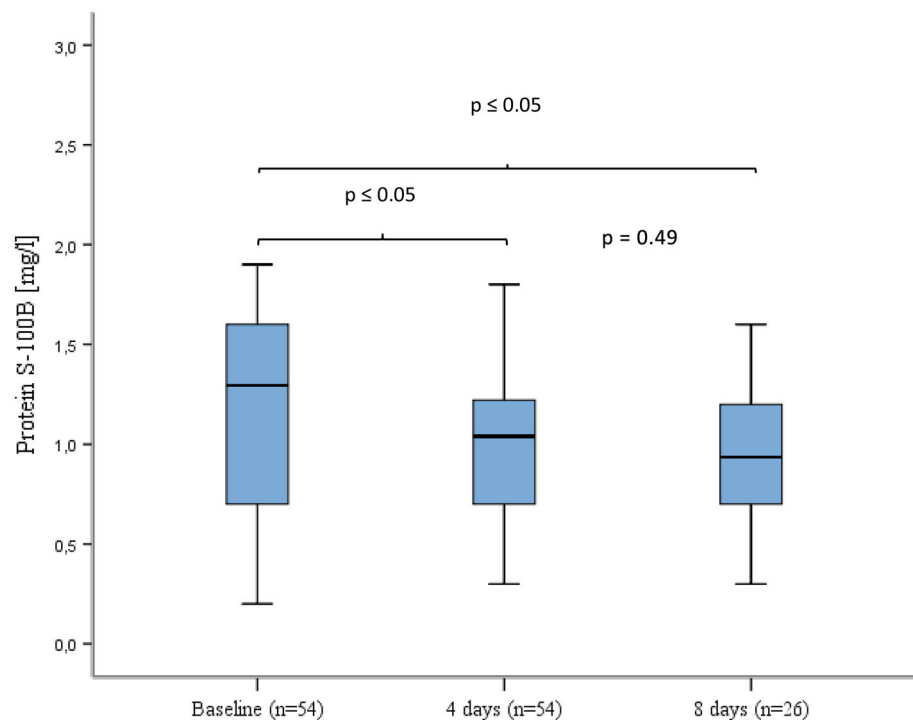
Besides the increases in tau protein levels (significant) and NfH^{SMI35} levels (non-significant), a decrease in S-100B levels was observed. Reduced astrocytic activity during TCA treatment may be due to corticosteroid-mediated immunosuppressive effects on microglial activation. As a result, the levels of interleukin-1 might decrease and lead to minor astroglial activation [34]. However, in vitro studies have shown that adrenocorticotropic hormone is responsible for increased secretion of S-100B [35]. At present, this phenomenon is not understood in detail. The neurotoxic and neuroprotective effects of S-100B protein have been discussed previously [36].

To understand the effects of TCA on astrocytic proliferation, further assessments are necessary. The effects of GCS on neuronal cells and on neuronal activity have been evaluated. A dose-dependent excitatory effect of methylprednisolone on cultured neuronal networks could be demonstrable [37, 38].

5 Conclusion

Intrathecal TCA therapy seems to be efficient and well tolerated for the treatment of symptoms in MS patients. Future treatment trials may investigate whether, in addition to symptomatic effects, TCA can reduce the inflammatory component of glia in the setting of severe transverse myelitis in MS and possibly also neuromyelitis optica spectrum disorders, and thus improve the clinical outcome. Neurotoxic side effects of GCS treatments have been

discussed for decades. The results of our study did not show neurotoxic effects; the levels of tau protein increased significantly but remained within the reference range. In addition, the level of S-100B protein decreased significantly during the first applications. Future long-term studies are needed to clarify the neurotoxic effects of TCA treatment.

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