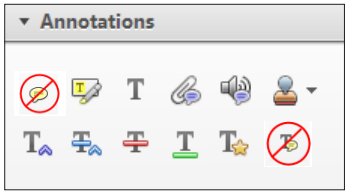
Page Proof Instructions and Queries

Journal Title: Therapeutic Advances in Neurological Disorders
Article Number: 636551

Greetings, and thank you for publishing with SAGE. We have prepared this page proof for your review. Please respond to each of the below queries by digitally marking this PDF using Adobe Reader.

Click "Comment" in the upper right corner of Adobe Reader to access the mark-up tools as follows:

For textual edits, please use the "Annotations" tools. Please refrain from using the two tools crossed out below, as data loss can occur when using these tools.



For formatting requests, questions, or other complicated changes, please insert a comment using "Drawing Markups."

Detailed annotation guidelines can be viewed at: http://www.sagepub.com/repository/binaries/pdfs/AnnotationGuidelines.pdf

Adobe Reader can be downloaded (free) at: http://www.adobe.com/products/reader.html.

No.	Query
	Please confirm that all author information, including names, affiliations, sequence, and contact details, is correct.
	Please review the entire document for typographical errors, mathematical errors, and any other necessary corrections; check headings, tables, and figures.
	Please ensure that you have obtained and enclosed all necessary permissions for the reproduction of art works (e.g. illustrations, photographs, charts, maps, other visual material, etc.) not owned by yourself. Please refer to your publishing agreement for further information.
	Please note that this proof represents your final opportunity to review your article prior to publication, so please do send all of your changes now.
	Please confirm that the Funding and Conflict of Interest statements are accurate.
1	Please provide a list of qualifications (MD, PHD, MSC, RN, etc.) For all authors
2	Is this Cu (II) or Cu^{+}?

Ther Adv Neurol Disord
1–5
DOI: 10.1177/1756285616636551

One-time intrathecal triamcinolone acetonide application alters the redox potential in cerebrospinal fluid of progressive multiple sclerosis patients: a pilot study

Thomas Müller, Thomas Herrling, Sven Lütge, Lutz Lohse, Gabi Öhm and Katinka Jung[AQ: 1]

Abstract

Introduction: Cerebrospinal fluid analysis may provide insight into the interplay between chronic inflammation and response to treatment.

Objectives: To demonstrate the impact of one intrathecal triamcinolone injection on the redox potential and on ascorbyl radical appearance in the cerebrospinal fluid of chronic progressive multiple sclerosis patients.

Methods: A total of 16 patients received 40 mg triamcinolone. Electron-spin resonance spectroscopy measured the oxidation range after copper ion [Cu (II)] addition and ascorbyl-radical bioavailability.

Results: There was an increase of Cu (II) ion absorption, which reflects an augmented content of reduced proteins. Ascorbyl radicals were present in contrast to healthy controls according to the literature.

Conclusion: Intrathecal steroid application alters the redox potential in cerebrospinal fluid. Our findings support the beneficial role of steroids on oxidative stress generally demonstrated by ascorbyl radical appearance. Reactive oxygen species decline is necessary for an upregulated production of reduced proteins.

Keywords: cerebrospinal fluid, electron-spin resonance spectroscopy, free radicals, multiple sclerosis, triamcinolone

Introduction

Multiple sclerosis (MS), as a chronic inflammatory disease of the central nervous system, is characterized by glial lesions that are monitored by MRI. Most patients finally suffer from a smouldering, chronic inflammatory, progressive MS process in the long term. Concomitantly, an upregulated free-radical synthesis may occur in the central nervous system [Ljubisavljevic *et al.* 2013; Mitosek-Szewczyk *et al.* 2010] that may be influenced by steroids [Seven *et al.* 2013]. Free radicals play a role in a variety of normal regulatory systems. Their dysregulation is an important component of chronic inflammation. Free radicals have the capacity to mediate tissue destruction, either alone, or in concert with proteases [Ljubisavljevic *et al.* 2013; Mitosek-Szewczyk *et al.* 2010]. Disturbances in free-radical-regulated second-messenger systems may contribute to the inflammatory process [Golde *et al.* 2003; Sellebjerg *et al.* 2002]. Cells and tissues have an abundance of antioxidant systems to scavenge or otherwise eliminate reactive oxygen species (ROS). Under normal circumstances, there is a well-managed balance between formation and neutralization of ROS [Aiken *et al.* 2011]. A potent mode of direct oxidative attack on a protein derives from site-specific metal-catalysed oxidation, that is, via

Correspondence to:
Thomas Müller
Department of Neurology, St. Joseph Hospital, Gartenstrasse 1, 13088 Berlin, Germany
th.mueller@alexius.de

Sven Lütge
Lutz Lohse
Gabi Öhm
Department of Neurology, St. Joseph Hospital Berlin-Weissensee, Berlin, Germany

Thomas Herrling
Katinka Jung
Gematria Test Lab GmbH, Berlin, Germany

protein-bound transition metals like copper (Cu^{+}). These processes generate active oxidative stress-related intermediates, such as superoxide anion, hydrogen peroxide or hydroxyl radicals. They oxidise, for instance, the chelating amino acids in proteins [Aiken *et al.* 2011; Mitosek-Szewczyk *et al.* 2010]. In the end, various oxidative mechanisms may modify the structure of proteins and cause loss of function, like in the case of oxidation of the plasma protein, fibrinogen, by treatment with an iron or ascorbate radical-generating system with its component C. This water-soluble vitamin reacts with several radical species, producing semidehydroascorbic acid or ascorbyl radicals. Here, the enzyme NADH-semidehydroascorbate reductase reduces ascorbyl radicals back to ascorbate, whilst oxidizing glutathione to its dimer, GSSG [Spasojevic *et al.* 2010]. In a healthy population, ascorbyl radicals were not found in cerebrospinal fluid (CSF), whereas they were elevated in amyotrophic lateral sclerosis, probably as a feature of the chronic neurodegenerative process [Spasojevic *et al.* 2010]. Generally, cells also possess oxidation–reduction–dependent repair pathways, which are triggered by oxidation of redox proteins. If cellular defending systems and repair processes fail, oxidatively damaged proteins can undergo direct chemical fragmentation or form large aggregates that may accumulate and disrupt important cellular processes. Therefore, timely removal of these lesioned proteins is important for maintenance of cellular homeostasis and viability [Aiken *et al.* 2011]. Failure of homeostasis ultimately causes apoptotic or necrotic cell death, both of which represent features of chronic progression in MS. From this point of view, it is interesting that intravenous methylprednisolone application beneficially altered substrates of upregulated free-radical peroxidation processes related to the pathophysiology of MS relapses [Mooradian, 1993]. Therefore, investigations of CSF and proteins that mediate the antioxidative potential in MS patients may provide some insight into the interplay between disease progression, chronic inflammation and response to treatment, such as intrathecal retarded-release steroid applications with triamcinolone (TCA) [Ljubisavljevic *et al.* 2013; Mitosek-Szewczyk *et al.* 2010; Müller, 2009; Sloka and Stefanelli, 2005]. The objective was to demonstrate the effect of one intrathecal TCA injection on the redox potential in the cerebrospinal fluid of chronic progressive MS patients in this observational pilot study.

Methods

Subjects

A total of 16 MS patients [age: 48.56 ± 8.72; MS duration: 11.23 ± 1.46 (mean ± SD, years); Expanded Disability Status Scale (EDSS): 6.34 ± 1.21; 10 women, 6 men; 10 secondary progressive (7 women and 3 men), and 6 primary progressive (3 women and 3 men)] patients were included. Exclusion criteria were an acute onset of exacerbation, or a recent clearly increased progression of their symptoms. The patients were free of relapses and of acute deterioration of symptoms.

Cerebrospinal fluid sampling and cerebrospinal fluid analysis

CSF was taken before the intrathecal TCA application. Aliquots of approximate 1 ml CSF were collected in sterile Eppendorf tubes. Within 2 hours, CSF analysis was performed. Samples were centrifuged to sediment eventual clouded material or cells. We transferred 35 µl CSF into a sterile reaction tube and added 5 µl H_2O_2 [Roth, Germany] 0.3% solution and 2 µl of the radical indicator, PCA (2,2,5,5 tetramethyl piperidine-N-oxyl) [Sigma–Aldrich, Germany], at 1 mmol. After vortexing, the reaction mixture was transferred into a capillary tube (haematocrit, 50 µl volume) [Hirschmann, Germany], closed on one end with Critoseal® wax and the electron-spin resonance (ESR) spectroscopy signal of the radical indicator PCA was detected by ESR on a Miniscope MS 200 Spectrometer [Magnettech Berlin, Germany] (parameters: 3363 G central field, 80 G scan range, 40 seconds' scan time, 0.14 seconds time constant, 1 G modulation amplitude, 7dB attenuation, 100 Gain). The capillary tube was then irradiated with UV radiation (Hönle SOL F2, 27.5 mW/cm^2 UVA and 5.4 mW/cm^2 UV B) for 1 minute. The ESR spectrum was again detected immediately after the irradiation. The free radicals are mostly hydrogen peroxides from the photocatalytic reaction. The oxidation range was determined by the addition of Cu (II) ions. A higher Cu (II) ion absorption reflects a higher amount of reduced proteins [Herrling *et al.* 2006; Jung *et al.* 2006]. The concentration of ascorbyl radicals was determined by ESR within 2 hours of performing lumbar puncture. Each CSF sample from every patient was analysed in duplicate. The means of both sample sets were employed for the statistical analysis.

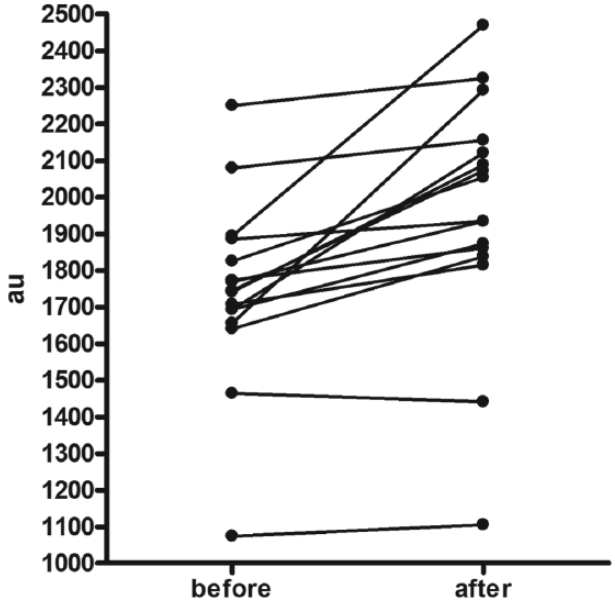


Figure 1. Increase of Cu (II) ion absorption determined before the first triamcinolone (TCA) administration and at the moment of the second cerebrospinal fluid sampling before the second TCA application.
Before, before the first triamcinolone (TCA) administration; after, after the first TCA administration; au, arbitrary units.

Design

The 40 mg TCA application was followed by a mandatory stay in bed for at least 8 hours in order to support and ease the diffusion of TCA in the CSF and the spinal cord. Only 2 days later, lumbar puncture was again performed with an 'atraumatic' Sprotte needle to apply the second TCA dosage. CSF was again taken before and transported to the laboratory at once. The pre-existing immune-system-modulating drug therapy remained stable. Spasticity-reducing therapy was not changed.

Statistics

Student's *t*-test for dependent samples was employed for the exploratory analysis of this pilot trial.

Ethics

All subjects gave written informed consent. An independent local institutional review board approved this study. The open observational study was advertised according to § 4 Abs. 23 Satz 3 AMG at the medical association. It was characterized as a noninterventional study, since serial CSF investigation during intrathecal treatment is an essential component of the routine associated with each lumbar puncture in daily clinical practice. It is also necessary to follow protein content and CSF-cell changes if inflammatory reactions in combination with the lumbar puncture occur.

Results

The second CSF assessment following the initial TCA administration showed an increase of Cu [AQ: 2] ion absorption, which reflects an elevated content of reduced proteins in the cerebrospinal fluid ($p < 0.0005$) (Figure 1). At the second determination, there was no significant decline of the ascorbyl radical levels (Figure 2). There were no relevant changes of protein content, cells and other concomitantly assessed routine parameters in CSF. The patients did not significantly improve after this one TCA application only (results not shown).

Discussion

Generally, intrathecal application of retarded-release steroids is under debate; not only in MS but also in other indications, that is, post-herpetic neuralgia or lumbar disk herniation [Kneissel, 1976; Kotani *et al.* 2000; Langmayr *et al.* 1995; Marinangeli *et al.* 2002]. To date, trials were mostly only observational and not placebo controlled due to ethical concerns. A certain spasticity-reducing and MS-process-improving effect is well known due to the observed, mean threefold increase of walking distance in progressive MS patients [Müller, 2009].

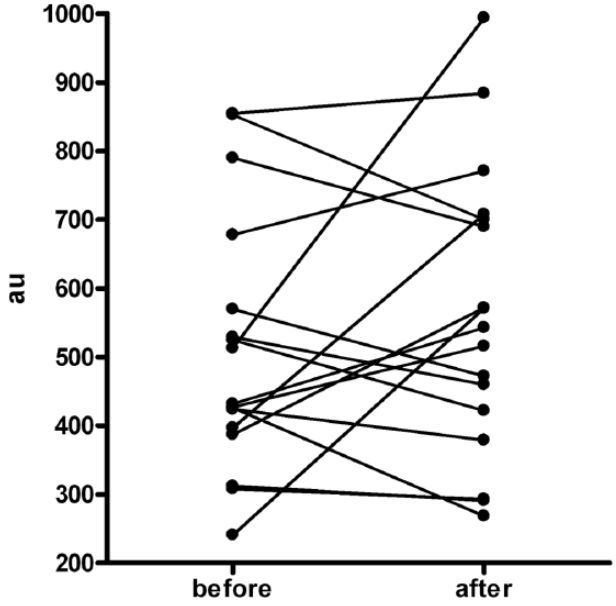


Figure 2. Ascorbyl radical levels before and after triamcinolone (TCA) application determined before the first TCA administration and at the moment of the second cerebrospinal fluid sampling before the second TCA application.
Before, before the first triamcinolone (TCA) administration; after, after the first TCA administration; au, arbitrary units.

Our experimental results suggest a potential anti-inflammatory effect due to a decreased free radical synthesis. Thus our findings support the beneficial role of steroids on oxidative stress, which is a feature of the acute and chronic smouldering inflammation in MS [Melcangi *et al.* 2000; Sellebjerg *et al.* 2002]. Decline of ROS is the prerequisite for the observed upregulated production of reduced proteins [Ljubisavljevic *et al.* 2013; Mitosek-Szewczyk *et al.* 2010]. Generally, it is known that free radicals play an important role in acute and chronic inflammation of the central nervous system in MS [Ljubisavljevic *et al.* 2013; Mitosek-Szewczyk *et al.* 2010]. The observed levels of ascorbyl radical concentrations in CSF seem to confirm this, since these radicals were not detectable in control CSF [Spasojevic *et al.* 2010]. However, this comparison with data from the literature must be viewed critically. There might be differences in terms of conditions of lumbar puncture and techniques of CSF analysis, etc. Our current outcomes do not allow any conclusion on the induction of putative regenerative mechanisms, which would be in line with the observed clinical benefit after repeat TCA therapy over an interval of at least 1 year [Müller, 2009]. But one should consider that oxidation–reduction–dependent repair pathways triggered by oxidation of redox proteins play an important role in proteasome metabolism, which is closely related to the improvement of cell viability, cell recovery, as well as the attenuation of oxidation-induced cytotoxicity associated with neuronal death in chronic neurological disorders [Aiken *et al.* 2011; Shacter, 2000]. Hypothetically, our findings suggest that an initial TCA application may modify via a free-radical-mediated signal-transduction pathway the function and regulation of regenerative proteins and growth factors. There are hints that TCA application may induce fall of soluble repulsive guidance molecule a (RGMa) fragments. This decline generally precedes neuronal recovery. One may even hypothesize that changes of free radical metabolism may trigger the observed descent of RGMa concentrations in patients with an enhancement of MS symptoms during TCA treatment [Müller *et al.* 2014, 2015]. However, this hypothesis warrants further investigation in combination with repeat clinical evaluation of MS patients, and assessment of further metabolic CSF substrates that are responsible for regenerative processes according to a similar signal-transduction pathway as in the case of fibrinogen generation [Aiken *et al.* 2011].

This observational pilot study has limitations. We did not perform our investigations in controls or in saline, respectively placebo-treated MS patients. Moreover, it is not ethical to obtain CSF from normal healthy controls for comparison of

the free radical status with the healthy condition. However, there is a need for further investigation; for instance, during acute relapse in larger cohorts of MS patients.

In conclusion, we show that intrathecal steroid application alters the redox potential in CSF.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare that there is no conflict of interest.

References

Aiken, C., Kaake, R., Wang, X. and Huang, L. (2011) Oxidative stress-mediated regulation of proteasome complexes. *Mol Cell Proteomics* 10: R110.

Golde, S., Coles, A., Lindquist, J. and Compston, A. (2003) Decreased iNOS synthesis mediates dexamethasone-induced protection of neurons from inflammatory injury *in vitro*. *Eur J Neurosci* 18: 2527–2537.

Herrling, T., Jung, K. and Fuchs, J. (2006) Measurements of UV-generated free radicals/reactive oxygen species (ROS) in skin. *Spectrochim Acta A Mol Biomol Spectrosc* 63: 840–845.

Jung, K., Richter, J., Kabrodt, K., Lucke, I., Schellenberg, I. and Herrling, T. (2006) The antioxidative power AP–A new quantitative time dependent (2D) parameter for the determination of the antioxidant capacity and reactivity of different plants. *Spectrochim Acta A Mol Biomol Spectrosc* 63: 846–850.

Kneissel, H. (1976) Treatment of lumbar disk herniation with depot cortisone intrathecally and peridurally. [Author's translation.] *Med Klin* 71: 1506–1507.

Kotani, N., Kushikata, T., Hashimoto, H., Kimura, F., Muraoka, M., Yodono, M. *et al.* (2000) Intrathecal methylprednisolone for intractable postherpetic neuralgia. *N Engl J Med* 343: 1514–1519.

Langmayr, J., Obwegeser, A., Schwarz, A., Laimer, I., Ulmer, H. and Ortler, M. (1995) Intrathecal steroids to reduce pain after lumbar disc surgery: a double-blind, placebo-controlled prospective study. *Pain* 62: 357–361.

Ljubisavljevic, S., Stojanovic, I., Pavlovic, D., Milojkovic, M., Sokolovic, D., Stevanovic, I. *et al.* (2013) Suppression of the lipid peroxidation process in the CNS reduces neurological expression of experimentally induced autoimmune encephalomyelitis. *Folia Neuropathol* 51: 51–57.

Marinangeli, F., Ciccozzi, A., Donatelli, F., Paladini, A. and Varrassi, G. (2002) Clinical use of spinal or epidural steroids. *Minerva Anestesiol* 68: 613–620.

Melcangi, R., Cavarretta, I., Magnaghi, V., Ciusani, E. and Salmaggi, A. (2000) Corticosteroids protect oligodendrocytes from cytokine-induced cell death. *Neuroreport* 11: 3969–3972.

Mitosek-Szewczyk, K., Gordon-Krajcer, W., Walendzik, P. and Stelmasiak, Z. (2010) Free radical peroxidation products in cerebrospinal fluid and serum of patients with multiple sclerosis after glucocorticoid therapy. *Folia Neuropathol* 48: 116–122.

Mooradian, A. (1993) Antioxidant properties of steroids. *J Steroid Biochem Mol Biol* 45: 509–511.

Müller, T. (2009) Role of intraspinal steroid application in patients with multiple sclerosis. *Expert Rev Neurother* 9: 1279–1287.

Müller, T., Barghorn, S., Lutge, S., Haas, T., Mueller, R., Gerlach, B. *et al.* (2015) Decreased levels of repulsive guidance molecule A in association with beneficial effects of repeated intrathecal triamcinolone acetonide application in progressive multiple sclerosis patients. *J Neural Transm* 122: 841–848.

Müller, T., Herrling, T., Lutge, S., Kuchler, M., Lohse, L., Rothe, H. *et al.* (2014) Reduction in the free radical status and clinical benefit of repeated intrathecal triamcinolone acetonide application in patients with progressive multiple sclerosis. *Clin Neuropharmacol* 37: 22–25.

Sellebjerg, F., Giovannoni, G., Hand, A., Madsen, H., Jensen, C. and Garred, P. (2002) Cerebrospinal fluid levels of nitric oxide metabolites predict response to methylprednisolone treatment in multiple sclerosis and optic neuritis. *J Neuroimmunol* 125: 198–203.

Seven, A., Aslan, M., Incir, S. and Altintas, A. (2013) Evaluation of oxidative and nitrosative stress in relapsing remitting multiple sclerosis: effect of corticosteroid therapy. *Folia Neuropathol* 51: 58–64.

Shacter, E. (2000) Quantification and significance of protein oxidation in biological samples. *Drug Metab Rev* 32: 307–326.

Sloka, J. and Stefanelli, M. (2005) The mechanism of action of methylprednisolone in the treatment of multiple sclerosis. *Mult Scler* 11: 425–432.

Spasojevic, I., Stevic, Z., Nikolic-Kokic, A., Jones, D., Blagojevic, D. and Spasic, M. (2010) Different roles of radical scavengers–ascorbate and urate in the cerebrospinal fluid of amyotrophic lateral sclerosis patients. *Redox Rep* 15: 81–86.