

# Reduction in the Free Radical Status and Clinical Benefit of Repeated Intrathecal Triamcinolone Acetonide Application in Patients With Progressive Multiple Sclerosis

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**Background:** Previous open trials performed repeated intrathecal application of the sustained release steroid triamcinolone acetonide every third day in patients with progressive multiple sclerosis and described enhanced walking abilities.

**Objectives:** The objectives of this study were to demonstrate the efficacy of 5 triamcinolone administrations every other day and to describe their effects on the amount of inducible free radicals in cerebrospinal fluid.

**Subjects/Methods:** Clinical ratings, determinations of maximum walking distance, and execution of an instrumental peg insertion test were performed at baseline and on each day after a triamcinolone injection in 21 patients with progressive multiple sclerosis. Induction of free radicals was assessed in cerebrospinal fluid before each triamcinolone application by electron spin resonance spectroscopy.

**Results:** Scores for multiple sclerosis improved, walking distance increased, and necessary intervals for the peg insertion procedure were shortened. The amount of inducible free radicals decreased.

**Conclusions:** Repeat triamcinolone application improves dysfunction of upper and lower extremities even when administered 5 times only and in series every other day. The declined potential for free radical synthesis may be caused by the anti-inflammatory effect of triamcinolone. It may contribute to suppress the smoldering, chronic inflammation, particularly in spinal lesions of patients with progressive multiple sclerosis. The enhanced arm function hypothetically reflects the effect on cervical and brain lesions due to the hypobaric features of triamcinolone.

**Key Words:** triamcinolone, multiple sclerosis, free radicals

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system. This disorder is characterized by various subtypes regarding its progression and its predominant cerebral and spinal localization of inflammatory lesions. In the long run, most patients end up in a smoldering, chronic inflammatory, progressive MS process with a concomitantly augmented nitric oxide-mediated free radical synthesis in the central nervous system. Free radicals play a role in a variety of normal regulatory systems on the one hand. On the other hand, their deregulation plays an important role during inflammation and its chronic progress. Free radicals may lead to the oxidative modification of low-density lipoproteins, the oxidative inactivation of  $\alpha$ -1-protease inhibitor, DNA damage/repair, and heat shock protein synthesis. At sites of inflammation, increased free radical activity is associated with the activation of the

neutrophil nicotinamide adenine dinucleotide phosphate-oxidase and/or the uncoupling of a variety of redox systems, including endothelial cell xanthine dehydrogenase. Free radicals, once produced, have the capacity to mediate tissue destruction due to chain reactions, either alone or in concert with proteases. Disturbances in the second messenger and regulatory activities of free radicals may also contribute to the inflammatory process. Release of nitric oxide, which promotes free radical synthesis, was elevated in the central nervous system of patients with MS. This metabolic change contributes to the progression of chronic inflammation and axonal demyelination.<sup>1–3</sup> Current mostly used 1.5- and 3.0-T magnetic resonance imaging techniques with gadolinium application may detect this kind of chronic smoldering inflammation with blood-brain barrier leakage particularly in the spinal cord only to a certain extent.<sup>4,5</sup> However, investigations of cerebrospinal fluid (CSF) and proteins, which mediate the antioxidative potential in patients with MS, may provide some insight in the interplay between disease progression, chronic inflammation, and response to treatment, such as intrathecal retarded release steroid applications.<sup>5,6</sup> Previous open trials performed repeated intrathecal application of the sustained release steroid triamcinolone acetonide (TCA) every third day in patients with progressive MS. These observational studies described an overall benefit with a distinct enhancement of walking abilities.<sup>6–11</sup> The objectives were to demonstrate the clinical efficacy of 5 repeated intraspinal TCA injections every other day on the upper limb function of patients with chronic progressive MS and to observe changes of the redox potential in their CSF in this observational pilot study.

## METHODS

### Subjects

Twenty-one patients with MS are included in Table 1. Exclusion criteria were an acute onset of exacerbation or a recent clearly increased progression of their symptoms. There was no evidence for an active inflammation according to a magnetic resonance imaging investigation (1.5 T, Siemens), which was performed before the start of TCA application. The participants were not allowed to receive steroids and were on a stable immunomodulatory drug regimen, if at all (interferon 1a [4 patients], glatiramer acetate [1 patient]) treatment for at least 4 weeks before study entry. They had to experience a distinct MS symptoms progression, which corresponded to at least 1 point on the Expanded Disability Status Scale, in the last 2 years before study entry but should be stable for at least 4 weeks before participation. The inclusion criteria did not allow participants with a history of seizures, subdural hematoma, and/or severe post-lumbar puncture syndrome.

### Design

Application of TCA was followed by a mandatory stay in bed for at least 6 hours to support and ease the diffusion of TCA in the

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**TABLE 1.** Demographic Features of the Participants

Age, mean (SD)	48.48 (2.72) y
Sex	11 women, 10 men
Secondary progressive	8 women, 7 men
Primary progressive	3 women, 3 men
Predominant spinal symptoms	4 women, 4 men
Predominant cerebral symptoms	2 women, 1 man
Spinal and cerebral symptoms	5 women, 5 men

CSF and the spinal cord. Lumbar puncture was performed with an “atraumatic” Sprotte needle. The preexisting immune system-modulating drug therapy remained stable. Spasticity-reducing therapy was not changed.

Ratings of EDSS, determinations of maximum walking distance, and execution of the instrumental test procedure were performed at baseline and on each day after a 40-mg TCA administration, dissolved in 10-mL saline.

### CSF Sampling and CSF Analysis

Cerebrospinal fluid was taken before the intrathecal TCA application. Aliquots of approximately 1 mL of CSF were collected in sterile Eppendorf tubes and frozen and stored at  $-20^{\circ}\text{C}$ . When the CSF analysis was done, samples were thawed and brought to room temperature ( $18^{\circ}\text{C}$ – $25^{\circ}\text{C}$ ). Then, they were centrifuged to sediment eventual clouded material or cells. Cerebrospinal fluid of 35  $\mu\text{L}$  was transferred into a sterile reaction tube. Five microliters of  $\text{H}_2\text{O}_2$  (Carl Roth GmbH + Co. KG Schoemperlenstr. 3-5, 76185 Karlsruhe Roth, Germany) 0.3% solution and 2  $\mu\text{L}$  of the radical indicator 2,2,5,5-tetramethylpyrrolidine-1-oxyl-3-carboxylic acid (PCA) Sigma-Aldrich Laborchemikalien GmbHWunstorferstr. 4030926 Seelze) at 1 mM were added. After vortexing, the reaction mixture was transferred into a capillary tube (hematocrit, 50- $\mu\text{L}$  volume, Hirschmann Laborgeräte GmbH & Co. KG Hauptstraße 7 - 15 · 74246 Eberstadt Germany), closed on one end with Critoseal wax, and the electron spin resonance spectroscopic (ESR) signal of the radical indicator PCA was detected by ESR on a Miniscope MS 200 Spectrometer (Magnettech, Berlin, Germany) (parameters: 33,631-G central field, 80-G scan range, 40-second scan time, 0.14-second time constant, 1-G modulation amplitude, 7-dB attenuation, 100 gain). The capillary tube was then irradiated with UV radiation (Hönle SOL F2, 27.5  $\text{mW}/\text{cm}^2$  UVA and 5.4  $\text{mW}/\text{cm}^2$  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. They interact with the radical indicator PCA with subsequent decrease of its signal intensity.<sup>12,13</sup> The amount of UV-induced free radicals is therefore directly determined by the measurement of the difference in the signal intensity of the PCA spin trap before and after UV radiation.<sup>14–16</sup> Each CSF sample of each patient was analyzed in duplicate. The means of both were used for the statistical analysis.

### Peg Insertion Procedure

The patients were asked to transfer 25 pegs (diameter, 2.5 mm; length, 5 cm) from a rack into one of 25 holes (diameter, 2.8 mm) in a computer-based contact board individually and as quickly as possible. The distance between the rack and the appropriate holes was 32 cm. The board was positioned in the middle, and the task was carried out on each side. When transferring each peg from the rack to the hole, elbows were allowed to be in contact with the table. The interval between inserting the first and the last pin initially with the right and then the left hand was measured with a

computer to 100-millisecond accuracy. The total peg insertion score is the sum of both intervals of task performance with the right and left hands in seconds.<sup>17</sup>

To minimize learning and training effects, we allowed to practice with the peg insertion instrument on the day before.

### Statistics

Analysis of covariance with repeated-measures design was used for this exploratory analysis of this pilot trial. Covariates were MS duration, MS types, sex, and age. The post hoc analysis was done with the Bonferroni multiple comparisons test against baseline.

### 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, paragraph 23, sentence 3 AMG at the medical association. It was characterized as a noninterventional study because 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

### Clinical Data

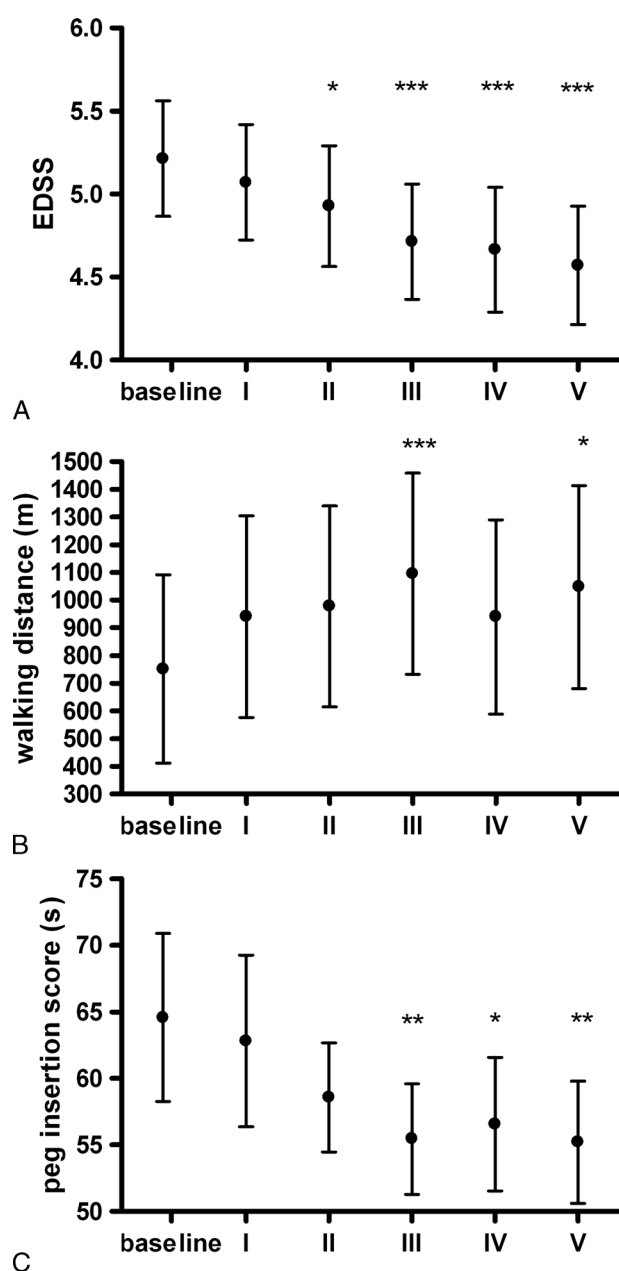
Score of EDSS improved ( $F = 14.17$ ;  $P < 0.0001$  [Fig. 1A]; maximum improvement,  $14.14 \pm 2.56$  [%; mean  $\pm$  SEM of the baseline value]), walking distance increased ( $F = 4.48$ ;  $P = 0.001$  [Fig. 1B]; maximum improvement,  $107.5 \pm 24.91$ ), and intervals for the performance of the peg insertion procedure were shortened ( $F = 5.83$ ;  $P < 0.0001$  [Fig. 1C]; maximum decrease,  $16.84 \pm 2.44$ ). No serious adverse effects appeared. No impact of the covariates was found.

### Laboratory Outcomes

There was a significant reduction of the induction potential of free radicals ( $F = 5.83$ ;  $P < 0.0001$  [Fig. 2]). The maximum reduction was  $45.76\% \pm 7.19\%$  (mean  $\pm$  SEM) of the baseline value.

## DISCUSSION

We confirm that repeated intrathecal TCA application improves MS symptoms and walking distance even when applied 5 times only and in series every other day.<sup>6–11</sup> The peg insertion outcomes show that this therapy may exert its benefit not only in the lower extremities but also in the upper limbs. Previous investigations always focused on walking distance and walking speed only.<sup>6</sup> A previous observational investigation, which compared the efficacy of repeated TCA application every 6 to 12 weeks over an interval of 1 year in 2 different cohorts with similar disease severity and symptom expression, demonstrated the superiority of TCA therapy over mitoxantrone treatment.<sup>9</sup> Another study and case reports confirmed this approach and the efficacy of reiterated TCA applications.<sup>10,11</sup> We emphasize that intervals between TCA applications and dosing of TCA dosing may vary in clinical practice. Triamcinolone acetonide therapy should be adapted to the individual symptom expression and response by the patient. We stress that this therapy should preferentially be performed in experienced centers.<sup>6</sup> This may also help to avoid onset of possible adverse effects such as post-lumbar puncture syndrome related to lumbar puncture or meningitis as consequence of a nonsterile TCA injection.<sup>6,18</sup>



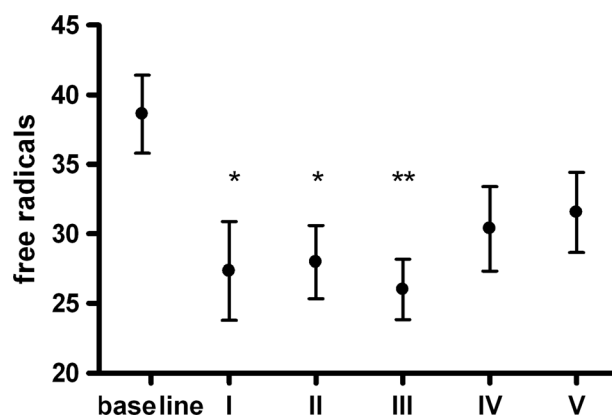
**FIGURE 1.** A to C, Clinical data on the response to repeated triamcinolone therapy. All data are given as mean  $\pm$  SEM; Asterisk indicates  $P < 0.05$ ; double asterisk,  $P < 0.01$ ; triple asterisk,  $P < 0.001$ ; asterisk, significance level of  $P$  values from the post hoc analysis; I, baseline before the first TCA administration; I, after the first TCA administration; II, after the second TCA administration; III, after the third TCA administration; IV, after the fourth TCA application; V, after the fifth TCA application; there was no assessment following the sixth TCA application because of technical reasons.

One may argue that high-dosage intravenous methylprednisolone application may be safer and similarly efficacious. There is only 1 study, to date, that tried to compare the efficacy of intravenous methylprednisolone administration with repeated intrathecal TCA injections, which were performed 3 times, at the most, only within 3 weeks.<sup>19</sup> No convincing superiority over the systemic

steroid treatment was found, but the design of this trial is deserving of criticism. For instance, enrolled patients with MS had had a recent deterioration in symptoms with acute relapses and/or ongoing chronic progression and were not classified according to the various subtypes of MS progression.<sup>19,20</sup> Generally, a comparison of both kinds of steroid application is objectionable because the resulting steroid efficacy in the central nervous system differs in favor for the intraspinal injection owing to higher CSF steroid levels and marked longer half-life with detection of TCA even 4 months after the last administration.<sup>20</sup> Intrathecal TCA administration did not decrease the endogenous peripheral cortisol secretion, which is a common adverse effect of oral or high intravenous methylprednisolone dosing. Long-term adverse effects, for instance, weight gain, osteoporosis, or skin alterations, were not observed in the context with intraspinal steroid application yet in contrast to trials on systemic oral and intravenous high steroid dosing in a repeated fashion in patients with MS.<sup>6,17,19–25</sup>

There was a certain, nonlinear variability of the clinical response to the TCA therapy, which may provide an up to 3-fold increase of maximum walking distance according to this and earlier trials.<sup>6</sup> We hypothesized that this behavior may result from a bidirectional TCA spreading in CSF to a certain extent.<sup>26–28</sup> Particularly, when injected into the CSF at the lumbar level, one may assume that most of the drug remains around the injection site. However, a large drug concentration gradient is created along the spinal cord, which may be responsible for a mechanism responsible for rostral TCA spreading dependent on baricity. As TCA is dissolved in saline directly before injection, the TCA solution becomes more hypobaric and lower than CSF density. Thus, lipophilic distribution against baricity may be supported particularly by the retard release steroid formulation in combination with penetration into neuronal cells along the spinal canal and thus probably in the brain in the long term.<sup>20,27,28</sup> Moreover, it is also known that bolus injection of hypobaric compounds also supports the spreading of applied compounds.<sup>29</sup>

Our experimental results from the laboratory suggest a potential anti-inflammatory effect due to reduction of free radical synthesis. Free radicals play an important role in acute and chronic inflammation of the central nervous system in MS. Thus, for instance, nitric oxide and related free radicals release resulting



**FIGURE 2.** Change of the free radical status in CSF under triamcinolone therapy. All data are given as mean  $\pm$  SEM; Asterisk indicates  $P < 0.05$ ; double asterisk,  $P < 0.01$ ; triple asterisk,  $P < 0.001$ ; asterisk, significance level of  $P$  values from the post hoc analysis; I, before the second TCA administration; II, before the third TCA administration; III, before the fourth TCA administration; IV, before the fifth TCA administration; V, before the sixth TCA administration.

from inflammation cause reversible conduction block in both normal and demyelinated central and peripheral nervous systems.<sup>3</sup> Accordingly, decrease of free radical synthesis may counteract neuronal damage.<sup>3</sup> This may result in improved neuronal function and movement capacity of the upper limbs as shown in this trial, for instance, with the peg insertion procedure, for the first time.

There are limitations. We did not perform this trial in controls or in saline and thus placebo-exposed patients with MS. Moreover, it is not ethical to obtain CSF from healthy controls for comparison of the free radical status to the healthy condition.

In conclusion, we show that repeated intraspinal steroid application improves clinical MS symptoms and dysfunction of the upper extremities. This result may be triggered by anti-inflammatory effects of the steroid with a concomitant downregulated free radical synthesis.<sup>3,30</sup>

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