● PERSPECTIVE

Biochemical indicators for neuronal regeneration during intrathecal triamcinolone application in multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). Essential characteristics are demyelination, inflammation and neurodegeneration. This process affects the white and grey matter in the CNS. MS patients experience various progression subtypes in association with the cerebral or spinal, acute inflammatory or glial sclerotic lesions (Müller, 2009). Most patients end up in a progressive, smouldering, chronic inflammatory process (Müller, 2009). Current predominantly used 1.5 respectively 3 Tesla MRI with Gadolinium® application visualize the various old and acute lesions. They serve as a biological marker in combination with standardised assessment of brain atrophy, black holes, *etc.* However, MRI with a stronger magnetic 7 Tesla field with better sensitivity gave hints on an ongoing, acute inflammatory, smouldering process even with Gadolinium® enhancing acute lesions in the brain and the spinal cord in progressive, so-called relapse free MS patients (Müller, 2009; Sinnecker et al., 2012). Additionally, progress of MS is determined with subjective standardised clinical ratings (Sinnecker et al., 2012). Both methods are used for the evaluation of the efficacy of relapse rate reducing drugs. These compounds, *i.e.*, interferons, teriflunamide, glatiramer acetate, fingolimod, fumarate or monoclonal antibodies, preponderantly weaken the malfunction of the peripheral immune system in relapse remitting MS patients. These MS drugs share one common disadvantage. They do not stop progression or improve MS within a framework of a regenerative process. They do not enable reversal of symptoms, for instance functional deficits or spasticity (Müller, 2009).

There is one exception. The repeat intrathecal application of the sustained release steroid triamcinolone acetonide (TCA) in primary and secondary progressive MS patients improves maximum walking distance, spasticity and occupational deficits of the upper extremities (Müller, 2009; Müller et al., 2014a, b). TCA interacts with the ongoing inflammatory process in the CNS by a slow but constant steroid release from crystalline structures. Accordingly, steroid cerebrospinal fluid (CSF) level after repeat application was found after an interval of 3 months (for review Müller, 2009). This treatment is performed in specialized centers in Germany for decades now. Observational trials in up to 161 patients reported benefits (Hellwig et al., 2004; Kamin et al., 2015). A comparison of this effect with sham saline intraspinal injections within a randomized, placebo controlled clinical trial design is not performed due to obvious ethical concerns yet (Hellwig et al., 2004; Müller, 2009).

TCA therapy is under debate, since it may be harmful due to occasional onset of post lumbar puncture syndrome, unspecific temporary inflammatory reactions within the CSF, sterile meningitis, constrictive arachnoiditis, cauda syndrome, CNS infections, therapy resistant headache, epilepsy, subarachnoidal and subdural bleeding, extreme elevation of CSF proteins (for review Müller, 2009). However patients respond, particularly when they suffer from spinal symptoms (Müller, 2009).

Clinically, there are three rough clusters of response to TCA applications in chronic progressive MS patients. Patients immediately improve during a series of four to six TCA applications or they show a delayed amelioration of disease symptoms after the TCA injections or they do not respond. Biochemical and pharmacological reasons for these three response patterns to TCA therapy are not known (Müller, 2009; Müller et al., 2014b). As a result of the low steroid dosing only, TCA therapy is rarely accompanied by onset of long term side effects, such as osteoporosis and weight gain, usually observed during the repeat high dosage intravenous steroid infusions for the treatment of acute relapses. There is now a certain resurgence of scientific research on this method of steroid application, which circumvents the blood brain barrier by the intrathecal application mode (Müller et al., 2014a, b; Rommer et al., 2014; Kamin et al., 2015). Biochemical CSF analyses during a series of TCA injections provided some circumstantial evidence that TCA may induce regenerative mechanisms. They may hypothetically support the observed enhancement of functional deficits. A reduced CSF production of free radicals was demonstrated in patients with a distinct immediate enhancement of upper and lower limb function during TCA therapy (Müller et al., 2014b). Free radicals play a role in a variety of normal regulatory systems, the deregulation of which is an essential component of inflammation. The physiological function of free radicals during inflammation includes the oxidative modification of low density lipoproteins, the oxidative inactivation of alpha-1-protease inhibitors, DNA damage/repair and heat shock protein synthesis. At sites of inflammation, increased free radical activity is associated for instance with the activation of the neutrophil NADPH oxidase and/or the uncoupling of a variety of redox systems, including endothelial cell xanthine dehydrogenase. Free radicals have the capacity to mediate tissue destruction, either alone or in concert with proteases. Disturbances in the second messenger and regulatory activities of free radicals may also contribute to the process of chronic inflammation (for review Müller et al., 2014b). Generally, free radicals also have the capacity to regulate production of various CSF proteins. There are molecules, such as neurotrophins, which initiate regenerative processes, such as remyelination and axonal recovery. There are also several inhibitors of regeneration physiologically existing in myelin and glial structures, *i.e.*, myelin-associated
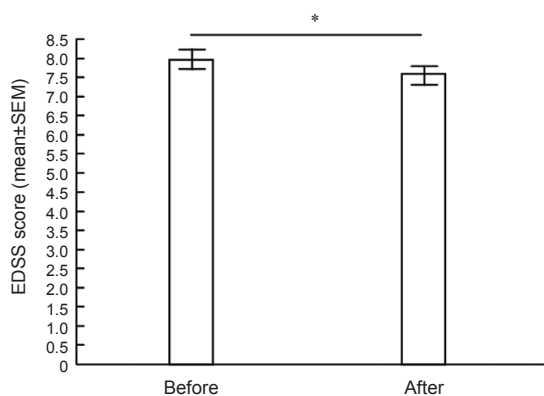
Figure 1 Reduction of the EDSS score.
*P-value < 0.05 of the Wilcoxon matched pairs test. SEM: Standard error of means; EDSS: Expanded Disability Status Scale; before/after: before/after intrathecal triamcinolone injections.
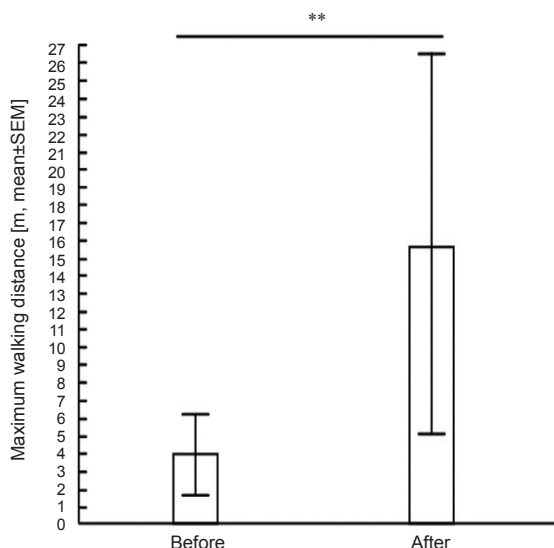
Figure 2 Increase of the maximum walking distance.
** P-value < 0.01 of the Wilcoxon matched pairs test. SEM: Standard error of means; m: meter; before/after: before/after intrathecal triamcinolone injections.

glycoprotein or repulsive guidance molecule A (RGMa). The glycosylphosphatidylinositol-anchored RGMa protein is processed by Furin and the proprotein convertase SKI-1 into numerous membrane-bound and soluble fragments. This processing is required for their proper *in vivo* functions. Several different fragments of RGMa exert their neurite growth inhibitory function by binding to their neuronal receptor Neogenin. Neogenin is a member of the immunoglobulin superfamily and consists of four N-terminal immunoglobulin-like domains (Ig), six fibronectin type III (FNIII) domains, a transmembrane domain and a C-terminal internal domain. Two different RGMa fragments, the N-terminal - (30 kDa) and the C-terminal fragment (40 kDa) bind to the same FNIII domain (domain 3–4) of Neogenin, despite their lack of sequence homology. RGMa exerts its neurite growth inhibitory function by binding to its neuronal receptor

Neogenin. This binding site is a member of the immunoglobulin superfamily. The encoded protein consists of four N-terminal immunoglobulin-like domains, six fibronectin type III domains, a transmembrane domain and a C-terminal internal domain. Neogenin is well known for its fundamental role in axon guidance and cellular differentiation. This binding site is also a dependence receptor functioning to control apoptosis. Basically, there are also two human RGMa variants, one with 40 kDa and one with 30 kDa according to western blot analysis. RGMa also modulates T cell responses. The RGMa receptor Neogenin is expressed by CD4^{+} T cells in humans. The RGMa-Neogenin interaction may be deregulated in immune cells and have a dual role in inflammatory neurodegenerative disease. Generally, Neogenin and RGMa are important regulators of cell death. Therefore, they may contribute to human chronic neurodegenerative processes for instance in MS and may prevent regeneration of damaged axons. In MS patients, RGMa is expressed by immature and mature dendritic cells in brain- and spinal cord lesions. Activated microglia cells express RGMa on their surface and decrease of microglial RGMa expression results in enhanced axonal growth both *in vitro* and *in vivo*. The RGMa gene was also identified as a disease-associated gene in MS patients and in certain rat strains induced with experimental autoimmune encephalomyelitis. *In vitro*, RGMa fragments inhibited neurite growth. Neutralization of RGMa activity with a polyclonal RGMa antibody in a spinal cord injury model resulted in long distance axon regeneration and improved functional recovery. Thus in conclusion, RGMa is a potent counteracting protein of neuronal regeneration and functional recovery and is accordingly well known as neurite outgrowth inhibitor in several animal systems and models of disease (for review Müller et al., 2014a). These findings suggest that RGMa plays a role in the regeneration failure in progressive MS patients. Recurrent TCA applications induced a decreased concentration of 30 and 40 kDA RGMa fragments (Müller et al., 2014a). This fall of soluble RGMa fragments may precede neuronal recovery and thus functional improvement, observed in these patients. One may even further hypothesize, that the observed free radical decline in CSF following repeated TCA injection may trigger this descent of RGMa concentrations in patients who experience an enhancement of MS symptoms during TCA treatment (Müller et al., 2014b). There was a certain, non linear variability of the clinical response to the TCA applications. This behavior may result from the TCA spreading in CSF, which is considerably influenced by two mechanisms. One depends on the CSF flow. Generally, CSF moves from the lateral ventricles, through the third and fourth ventricle, into the subarachnoid space around the brain and the spinal cord. CSF does not move in a one directional manner. CSF is moved in a pulsatile way, synchronous with the contraction of the heart. During each systole blood is pumped into the cerebral arteries, causing an increase of the intracranial volume.

Since brain and blood are not compressable, some CSF will be forced in a caudal direction into in the spinal canal, which is not expandable since it is not constricted by the skull (Nakamura et al., 1998). This CSF flow reverses in a rostral way during the diastole. A physiological model on CSF dynamics calculated that this process displaces only 0.5–2 mL of CSF with every heartbeat. At the spinal level pulsations from the spinal arteries contribute to these pulsatile waves as well. Since the driving force of these pulsatile movements starts in the skull, its effect decreases caudally, resulting in a limited CSF flow at the lumbar/low thoracic level (Nakamura et al., 1998). When injected into the CSF at this level, most of the drug remains around the injection site. Accordingly, a large drug concentration gradient is created along the spinal cord, which may be responsible for the second 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 (Müller, 2009; Hejtmanek et al., 2011). Thus lipohilic distribution against baricity is supported by the retard release of the steroid in combination with penetration into neuronal cells along the spinal canal and probably also in the brain in the long term. Thus, both mechanisms may counteract a possible drug effect only in the vicinity of the lumbar injection site. Moreover it is known that bolus injection of hypobaric compounds also supports the spreading of applied compounds (Hejtmanek et al., 2011). There are also concerns that this slow steroid release from crystalline structures is harmful to neuronal and glial cells (for review Müller, 2009). CSF investigations with markers for neuronal damage or death, such as neuron specific enolase, S-100, neurofilament heavy-chain, tau protein provided no convincing evidence in favor of these hypothetical concerns (Hellwig et al., 2006; Rommer et al., 2014).

To date, current observational trials only included patients with an Expanded Disability Status Scale (EDSS) score described effects in patients within a median EDSS range between 5 and 7 (Müller, 2009). Following the initial series of repeat intrathecal TCA application (mostly six injections with 40 mg TCA each), it is advisable to repeat this procedure every 6 to 12 weeks once or twice dependent on the condition of the patient (Hellwig et al., 2006; Müller, 2009). The current promising clinical and biochemical outcomes now justified a further observational investigation on the effect of this therapy in more advanced MS patients with an EDSS score higher than 7, who can barely walk. Nine patients (5 men, 4 women, age: 52.11 ± 2.55; 43 – 65 years [mean ± SEM; range]), received six TCA application with a distinct higher, cumulative total TCA dose of 346.7 ± 42.82; 240 – 560 mg within 14 days every second day. **Figure 1** shows the significant (Wilcoxon matched pairs test: $P = 0.016$) reduction of the EDSS score, and **Figure 2** shows the significantly ($P = 0.008$) increased maximum walking distance. These preliminary clinical results demonstrate that higher

TCA dosing may even induce clinical benefits in more severely affected chronic MS patients. These outcomes warrant further research in the clinic and in the laboratory to understand the clinical response and the possible mechanisms of repeat TCA applications.

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