Toxicity of CSF in motor neurone disease: a potential route to neuroprotection

The paper by Tikka and colleagues in this issue of *Brain* reports that CSF from patients with motor neurone disease (MND) is toxic to cultured spinal cord neurones and that the tetracycline antibiotic minocycline exerts a significant neuroprotective effect against this toxicity (Tikka et al., 2002).

There is a tendency in MND for the disease to start focally, for example with weakness of one hand, unilateral foot drop or dysarthria. As judged by clinical signs of progression, the disease typically tends to spread to contiguous groups of motor neurones. There has been great interest in the possible diffusible factors which may contribute to this propagation of neuronal injury between adjacent groups of motor neurones. Potential candidates include nitric oxide; free radical species or toxic chemicals resulting from free radical reactions such as 4-OH nonenal; molecules released from activated microglia, as well as an increase in the extracellular level of the excitatory neurotransmitter glutamate. The toxic factors may be detectable in CSF, the composition of which reflects changes in the chemistry of the extracellular space in the CNS.

There have been several previous studies that demonstrate the toxicity to neurones in culture of the CSF from MND patients (Terro et al., 1996; Couratier et al., 1993; Manabe et al., 1999). CSF toxicity has also been reported in other neurodegenerative disorders, for example, Hao and co-workers reported that CSF from patients with Parkinson’s disease selectively inhibited the growth and function of dopaminergic neurones in culture (Hao et al., 1995). Exposure to MND CSF has also been shown to increase the abundance of phosphorylated neurofilaments in the cell body of cultured motor neurones (Nagaraja et al., 1994). Increased levels of phosphorylated neurofilaments in motor neurone perikarya have also been reported in the spinal cords of MND patients and Cu/Zn superoxide dismutase (SOD1) mutant transgenic mice. These findings, taken together, are consistent with the possibility that constituents of CSF may be responsible, at least in part, for motor neurone pathology in MND.

Several potential candidates for the mysterious ‘toxic factor’ have been identified from studies of CSF neurochemistry in MND. For example, several groups have reported that the level of glutamate in CSF is significantly raised in at least a subgroup of MND patients, though not all laboratories have confirmed this finding. Failure of the normally tight regulation of the level of extracellular glutamate may result in excitotoxic effects on neurones. The level of 4-hydroxy-nonenal (HNE), a marker of lipid peroxidation, was reported to be >3-fold elevated in MND patients compared to controls (Smith et al., 1998). Such an increase in the level of HNE was sufficient to kill motor neurone hybrid cells in vitro, and antioxidants prevented this HNE-dependent cell death. Toghi and colleagues reported a mean 7-fold increase in the concentration of 3-nitrotyrosine, a biochemical marker indicating increased formation of the highly damaging peroxynitrite (ONOO·) radical, in the CSF of MND patients compared to controls (Toghi et al., 1999). Neurofilament light protein has been reported to be increased in the CSF of MND patients (Rosengren et al., 1996) and it is possible that cytosolic proteins may exert toxic effects, when released from dying motor neurones into the extracellular space and CSF. Finally, the proliferation of non-neuronal cells, including astrocytes and microglia, seen in pathologically affected areas within the CNS of MND patients, may lead to an imbalance of neurotrophic and neurotoxic signalling in the vicinity of motor neurones. Activated astrocytes and microglia are known to release a variety of potentially neurotoxic factors such as free radicals, glutamate, cytokines, chemokines and metalloproteases (Ridet et al., 1997; Gonzalez-Scarano et al., 1999). Several drugs including AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor antagonists (Couratier et al., 1993), riluzole and anti-oxidants (Terro et al., 1996) have all been shown to exert a neuroprotective effect against the toxicity of CSF from MND patients in experiments with cultured neurones. Minocycline, a semi-synthetic derivative of tetracycline and a widely used antibiotic, is an inhibitor of microglial activation. Minocycline has been shown to exert beneficial effects in animal models of MND (Robberecht et al., unpublished observations) as well as Huntington’s disease and Parkinson’s disease (Chen et al., 2000; Du et al, 2001).

In the study reported by Tikka and colleagues in the current issue of *Brain* (Tikka et al., 2002), CSF from three groups of MND patients was found to be toxic to cultured spinal neurones when compared with the effects of CSF from patients with other neurological diseases. The three groups consisted of patients with sporadic MND, patients homozygous for the D90A SOD1 mutation and patients with non-SOD1 related familial MND. Cellular toxicity was assessed by measurement of LDH (lactate dehydrogenase) release into the culture medium, Hoechst 33342 staining to detect
apoptotic nuclei and neuronal counts following NeuN staining. Immunocytochemistry was also used to assess microglial proliferation and neurofilament phosphorylation within neurones. The CSF samples were separately analysed for the levels of glutamate, aspartate and glycine; immunoreactivity against spinal cord proteins and the activity of matrix metalloproteases (MMPs) 2 and 9.

The CSF from all three groups of MND patients was found to trigger increased apoptotic cell death and decreased neurofilament phosphorylation of spinal cord neurones, together with increased microglial activation. There was an ~5-fold increased detection of apoptotic nuclei in spinal cord neurones when exposed to CSF from MND patients compared to exposure to control CSF. Neuronal cell death, but not microglial activation, was prevented by NMDA (N-methyl-D-aspartate) and AMPA receptor antagonists, whereas administration of minocycline to the cultures prevented both microglial activation and neuronal death. It is clear that some details of the toxicity observed with MND CSF vary with the experimental paradigm used. For example, the study by Tikka and co-workers demonstrated reduced phosphorylation of neurofilament proteins expressed within the cell body of spinal neurones and also significant neuroprotection by NMDA as well as AMPA antagonists. Some other studies have reported differing results for these two parameters. Tikka et al. found that the toxicity of MND CSF was not accounted for by alteration in the level of excitatory amino acid neurotransmitters, immunoreactivity against proteins present within spinal neurones or increased activity of MMPs. The authors concluded that release from microglia of factors that increase toxic effects resulting from glutamate receptor activation may account for the observed CSF toxicity.

In other experimental settings glial cells have been shown to play a role in excitotoxic neuronal death and there are at least three potential mechanisms (Ridet et al., 1997; Gonzalez-Scarano and Baltuch, 1999). (i) Astrocytes can release glutamate in the vicinity of neurones by a Ca*-dependent mechanism. (ii) Activated microglia secrete tumour necrosis factor alpha (TNF-α) which has been shown to enhance AMPA mediated excitotoxicity in cultured cortical neurones. (iii) Activated microglia release MMPs, which can exert neurotoxic effects via activation of pro-inflammatory molecules or through an effect on the extracellular matrix and interference with integrin signalling.

The interesting study by Tikka and co-workers is important in several ways. First, it has demonstrated that similar toxicity is present in CSF from patients with sporadic and genetic forms of MND, supporting the notion that common pathophysiological mechanisms contribute to the propagation of motor neurone injury, even though the upstream disease initiating process may differ. Secondly, they provide evidence that a readily available and safe drug, minocycline would be a useful agent to test therapeutically in patients with MND. Finally, this study provides evidence that monitoring of CSF neurotoxicity could be a useful way of evaluating the neuroprotective effects of new therapeutic agents in the future.

References


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