Journal of Neurochemistry

JOURNAL OF NEUROCHEMISTRY | 2016 | 137 | 140–141 doi: 10.1111/jnc.13296

EDITORIAL HIGHLIGHT

CAST your vote: is calpain inhibition the answer to ALS?

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Read the highlighted article *'Calpastatin inhibits motor neuron death and increases survival in hSOD1^{G93A} mice' on page 253.*

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that affects motor neurons in the brainstem, spinal cord, and motor cortex, resulting in muscle atrophy and weakness. The majority of ALS cases appear to be idiopathic, while 5–10% of ALS cases are presumed to be due to familial onset. Among these genetic causes, 20% are due to mutations to the superoxide dismutase 1 (SOD1) gene, which causes misfolding of the antioxidant enzyme and selective degeneration of motor neurons by unknown mechanisms (Robberecht and Philips 2013). Presently, the only drug available for patients is riluzole, which is believed to prevent glutamate release and inhibit postsynaptic glutamate receptor signaling, thus blocking excitotoxicity (Hunsberger *et al.* 2015). The causes of neuronal loss in ALS are, however, still unclear, and consequently an extensive worldwide search for the underlying degenerative mechanism is underway.

In a recent paper published in the *Journal of Neurochemistry*, Rao *et al.* (2016) investigated the role of calpastatin (CAST), an endogenous calpain inhibitor, in a low expressing hSOD1^{G93A} mouse model of ALS. Calpains are activated in response to elevated calcium levels, possibly through increased glutamatergic neuronal transmission, and are hypothesized to contribute to neurodegeneration via necrotic and apoptotic pathways (Vosler *et al.* 2008). In the present study, Rao *et al.* (2016) detected increased calpain activation and reduced levels of CAST in the spinal cord of SOD1^{G93A} mice at a late stage of the disease, implicating calpains in neuronal death in ALS. This is particularly interesting, given that excitotoxic cell death is present in ALS and calpains are thought to have a role in excitotoxic neurodegeneration.

To investigate whether calpain inhibition is beneficial to the ALS phenotype, Rao *et al.* (2016) crossed a transgenic mouse that overexpresses CAST under the Thy-1.1 neuronal promoter with a low level expressing hSOD1^{G93A} mouse. The authors found that hSOD1^{G93A} mice that overexpressed CAST had a significant increase in the number of surviving motor neurons at the later stages of disease. Subsequently, the authors demonstrated a delay in disease onset and a significant increase in survival days in hSOD1^{G93A} mice. Biochemically, the overexpression of CAST in hSOD1^{G93A} mice increased expression of several neurofilament proteins indicating restoration of neuronal signaling through calpain inhibition. Further, the authors revealed that overexpression of CAST led to small reductions in SOD1 oligomers (Rao *et al.* 2016). This is an interesting observation, given the emerging evidence of the toxic nature of SOD1 oligomers (Wang *et al.* 2006). Collectively, these findings lead to the suggestion that calpain inhibition may be a therapeutic target in ALS.

More than 50 endogenous and exogenous inhibitors of calpain have been described and these inhibitors have possible therapeutic applications to a range of disorders where neuronal cell loss has been implicated. Indeed, Phase 1 clinical trials are already underway with compounds that inhibit calpains in Alzheimer's disease. However, several factors must be carefully assessed prior to considering calpain inhibitors as a therapeutic target for ALS:

First, as with several common neurodegenerative disorders, mouse models of ALS are based on mutations that occur in a small subset of the respective disease population.

Received July 28, 2015; accepted August 14, 2015.

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Abbreviations used: ALS, amyotrophic lateral sclerosis; CAST, calpastatin.

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Indeed, approximately 90% percent of ALS cases are sporadic and the origins of onset in many instances of the disease are still unknown. Caution is thus required when interpreting results from models based on SOD1 mutations. The need for such caution has been highlighted by previous investigations, which showed positive effects of a treatment in mutant SOD1 models, though this did not translate into clinical trials (Genç and Özdinler 2014).

Second, in the Rao *et al.* (2016) study, calpain is inhibited by overexpression of CAST from birth. In humans, however, treatment usually begins only after diagnosis, which can occur sometime after disease onset. Future studies must focus on the ability of CAST and other calpain inhibitors to be given in the later stages of disease onset.

Third, a detailed preclinical assessment is necessary to determine the impact of calpain inhibition on preventing disease onset in several ALS models. The mouse model employed by Rao *et al.* (2016) has a low copy number of $hSOD1^{G93A}$ and is a less aggressive model than is often used in the literature to assess treatments. Future studies may utilize other more aggressive models and/or non-SOD1 mutation models of ALS to further draw conclusions as to the correlation between calpain inhibitors and ALS lifespan.

Fourth, quantification of calpain expression in human ALS tissue would be of value for understanding the involvement of calpain in motor neuron loss.

These above preclinical studies might provide clues as to the potential and capacity of calpain inhibition to be therapeutically beneficial to ALS patients.

Finally, a key question of interest is the extent to which motor neuron expression of calpain contributes specifically to the disease compared to its effects in other cell types. In the present study, the Thy-1.1 promoter used to drive CAST overexpression increased expression in several neuron types, limiting the ability to determine if the effects of inhibiting calpain are motor neuron specific. Future studies that utilize a motor-neuron-specific-promoter are required to determine the beneficial effects of CAST overexpression to motor neurons that degenerate in ALS. Furthermore, mechanisms leading to calpain activation are not yet fully elucidated and other cell types may be involved. Previous investigations by Rao *et al.* (2008) and others (Liang *et al.* 2010) have shown in other degenerative models that neuronal CAST overexpression has some anti-inflammatory effects through reduction to GFAP-positive astrocytes (Rao *et al.* 2008), and the normal physiological role of calpain/CAST remain largely unknown. Investigations using CAST expression in multiple cell types may provide some insight into the role of calpain expression in different cell types so as to address this question.

On average, ALS leads to death within 3 years of symptom onset and there is now an urgent need for a deeper understanding of ALS pathology. The investigations by Rao *et al.* (2016) have provided intriguing evidence that calpain inhibition can lead to motor neuron protection in an ALS mouse model. Calpain activation has been implicated in several other neurological diseases, including Alzheimer's disease, Parkinson's disease, stroke and brain trauma and therefore the authors suggest a possible common neurodegenerative mechanism shared by several adult onset brain disorders. Further research into early activation and cell signaling dysfunction in ALS will highlight the mechanisms driving protection through CAST overexpression and its relevance to the disease.

Acknowledgments

This work was supported by National Health and Medical Research Council (NHMRC) of Australia via research project grant #1083569, as well as fellowships from the Roth Foundation, Spinal Cure Australia and a grant from Neill Miller. The authors would also like to thank Chris Vaughan for discussion of this work. BV is an editor for the Journal of Neurochemistry.

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