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Commentary

Calpain Inhibition as a Potential Treatment of Alzheimer's Disease

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Alzheimer's disease is a debilitating, progressive, chronic inflammation that influences memory defects and cognitive decline. Although there are some examples of early Alzheimer's disease associated with single-gene abnormalities, the vast majority of patients with the disease exhibit sporadic, perhaps multigenic, pathogenesis. Its frequency increases with age. Thus, in industrialized societies, the frequency of Alzheimer's disease is increasing and is posing a major burden on families and society. Attempts to reverse the cognitive decline of Alzheimer's disease have met with very limited success, although some experimental procedures and clinical trials now being considered seem to offer some promise. There are two reasons for this relative lack of success—the difficulty in making unequivocal diagnoses in patients during the early phases of the disease, and the paucity of agents that produce a clearly favorable outcome.

Amyloid-β

One of the pathological features of Alzheimer's disease is the presence of amyloid plaques, composed of amyloid-β (Aβ) and other proteins, in specific regions of the brain as well as dystrophic neurites. Aβ is generated in an orderly sequence of proteolytic events, catalyzed by β-secretase and γ-secretase, acting on the transmembrane amyloid precursor protein (APP) producing the hydrophobic Aβ peptide that self-aggregates to generate oligomers and larger aggregates (amyloid) (reviewed by Querfurth and LaFerla[1]). On the other hand, cleavage of APP by α-secretase and γ-secretase generates products that differ from Aβ and are thought to be nontoxic.

There has been much debate about the precise nature of the toxic Aβ species, although prevailing opinion seems to favor soluble (paucimolecular) aggregates. The steady-state levels of Aβ in various susceptible locations of the brain (eg, hippocampus and frontal cortex) are determined not only by the substance's generation rate but also by its degradation or clearance rate. As it is known that the accumulation of Aβ is the critical factor associated with the development of cognitive decline, it is not unexpected that the focus of most therapeutic approaches are aimed at the reduction of the steady-state levels of Aβ and its toxic aggregates. Among the most commonly used approaches are vaccination with Aβ, treatment with monoclonal antibodies against Aβ, interference with β-secretase activity, the enzyme critical for Aβ generation, inhibitors of Aβ aggregation, nerve growth factors, etc. The pathophysiological mechanisms that influence the outcome of these approaches are confounded by the multiple conformers and aggregates of Aβ.

Calpain and Alzheimer's Disease

Various Aβ conformers contribute to cytotoxicity in the Alzheimer's disease brain, but how does this occur? Few of the approaches hitherto taken have been directed at the downstream influences of Aβ signaling and toxicity. This is what makes the report from the LaFerla laboratory in this issue of *The American Journal of Pathology*[2] of special interest. These investigators studied the use of a novel calpain inhibitor, A-705253, which can be readily administered in drinking water. Aβ alters the calcium homeostasis of neurons by interactions with voltage-gated calcium channels and perhaps with the *N*-methyl-D-aspartate receptor. Increased cellular calcium levels activate the calcium-dependent protease calpain.

Calpains are widely distributed, calcium-activated neutral proteases that have a multitude of functions. The most studied forms of calpain are the m and μ calpains. However, although 12 additional calpain cDNAs have been identified,[3] only a few have been studied in detail. The m and μ calpains are heterodimers composed of a precursor of the active protease (80-kDa molecular weight) and a 28-kDa regulatory subunit. The 80-kDa precursor is autocatalytically processed to 78- and 76-kDa active products. Although these enzymes are cal-

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cium activated, the physiological levels of intracellular calcium do not correspond to the optimal calcium requirements for calpain activity, the so-called calcium problem despite the lowering of calcium requirement by phospholipids. The role of calcium homeostasis in Alzheimer's disease has recently been reviewed.[4] The transport of calcium from endoplasmic reticulum stores to the cytosol is mediated by several transporters.

An endogenous network, constituted of calpains and their inhibitor calpastatin, exists to preclude uncontrolled overactivation of calpain. The significance of this network is suggested by findings in the central nervous systems of patients with Alzheimer's disease. The widespread activation of calpains, as reflected in the ratio of active protease fragment (76 kDa) relative to precursor, was noted in the brains of Alzheimer's disease patients.[5] Additionally, calpastatin was reported to be depleted in Alzheimer's brains with consequent calpain activation.[6] The increased activity of calpains in the brains of Alzheimer's disease patients is also suggested by the high level of calpain-cleaved spectrin found in the cerebrospinal fluid (CSF) of these patients.[7]

Calpain Inhibition in Murine Alzheimer's Disease Models

The potential importance of the regulation of calpain activity in experimental models of Alzheimer's disease is evidenced by studies involving the overexpression of calpastatin.[7] This overexpression results in a reduction of amyloid burden, tau phosphorylation, and microgliosis. The reverse is the case in calpastatin-knockout animals. Of interest, this interdiction of calpain activation by increasing calpastatin does not result in any change in body weight, longevity, synaptic markers, or cytoskeletal components. In addition, marked motor or behavioral impediments are not observed in this model. These results indicate the importance of the absence of notable side effects with the constitutive inhibition of calpain activity. If calpain inhibition is to be considered as a potential long-term therapeutic treatment, it is essential to establish that there are no serious side effects of this inhibition.

In the studies of Medeiros et al,[2] the calpain inhibitor A-705253 was administered to mice in an Alzheimer's mouse model associated with the generation of increased levels of both Aβ40 and Aβ42 due to the overexpression of the Swedish mutation of APP and a presenilin mutant (M146V) knockin, and increased neurofibrillary tangles, another pathological feature of Alzheimer's disease, due to the expression of a tau mutant (P301L), which is more readily phosphorylated.[8] The presenilin protein forms an active proteolytic complex with β-secretase. In addition, presenilin may also influence endoplasmic reticulum calcium transport. A-705253 is a benzoylalanine-derived ketoamide[9] that has been previously shown in primary embryonic hippocampal neurons to inhibit Aβ-induced cleavage of dynamin 1 and tau,[10] as well as Aβ-induced synaptic dysfunction in hippocampal slices and primary neuronal cultures.[11,12] *In vivo*, it has been shown to decrease Aβ-induced behavioral dysfunction in rats.[12] In the study by Medeiros et al,[2] the calpain inhibitor was given orally to these mice every day between the ages of 15 and 18 months at two doses, 40 and 80 mg/kg. Untreated transgenic mice exhibited behavioral deficits as evidenced by their performance in the hippocampus-dependent Morris water maze test, in the cortex-dependent novel object recognition task, and in the contextual fear conditioning task. In each of these tasks, the treated animals exhibited a marked dose-dependent improvement. At high inhibitor concentrations, the animals behaved as well as the control non-transgenic mice. As with the transgenic calpastatin model, there were no significant changes in learning or memory functions in the inhibitor-treated non-transgenic control mice, and no changes in the motor functions as evidenced by movements associated with the behavioral tasks, again suggesting that the inhibitor did not engender serious side effects despite the widespread expression of calpain. Although the off-target effects of calpain inhibition appear to be minimal in this medium-range duration of treatment, it remains to be established whether there might be unfavorable influences after longer-term treatment. This said, such effects might not be critical, given the nature of the disease being treated. However, it is worth noting that these experiments were conducted in mice in controlled pathogen-free environments in which the effects of chronic stresses are minimized. Calpain activation may be a common response to injury and may be a downstream effector of growth factor stimulation. For example, calpain appears to mediate the activation of TGFβ in rodent models of pulmonary hypertension.[13] Calpain inhibition also impairs insulin release from pancreatic β cells.[14] Thus, inhibition of calpains may well have untoward effects in free-living environments.

In A-705253-treated transgenic mice, there was a notable reduction in the number and size of amyloid plaques in the brain parenchyma that correlated with the reduced level of Aβ measured biochemically. This finding was apparently related to a reduction in β-secretase and an increase in ABCA1 transporter. The reduction in the former reduces the production of Aβ, and others have noted a similar regulation of β-secretase levels by calpain manipulation.[15] ABCA1 has been suggested to promote the clearance of Aβ.[16] In this case, the transporter level was posttranslationally regulated via inhibition of the calpain-mediated proteolytic degradation of ABCAI involving the PEST sequence in the transporter.[17] Consistently, LXR, the nuclear hormone receptor that primarily regulates ABCA1 transcription, is not changed by calpain modulation. No changes were observed in the amyloid-degrading enzymes microglial neprilysin and insulin-degrading enzyme, or in apoE levels. Parenthetically, in another study, the use of an LXR agonist resulted in enhanced degradation of Aβ mediated by the induction of lipidated apoE, resulting in the promotion of Aβ degradation via these pathways.[18]

The tau hyperphosphorylation was also reduced in the Medeiros et al experiment, apparently due to the attenuated conversion of the CDK5 activator p35 to its p25 form, a calpain-dependent transformation, resulting in a

decreased activation of CDK5. Along with the behavioral improvement, there was an enhancement in synaptic function as measured by the levels of presynaptic synaptophysin and postsynaptic PSD-95. The final examined pathological feature altered by calpain inhibition was the neuro-inflammatory response, with decreasing numbers of degenerating neurons, astrocytes, and microglia in contrast to what was observed in vehicle-treated transgenic mice.

This is not the first example of the effect of inhibition of calpain by "drug" therapy in experimental models of Alzheimer's disease that result in protection against developing features of neuropathology and behavioral deficits. Using two different calpain inhibitors, E-64 and a more specific inhibitor BDA-410, Trinchese et al[19] noted similar overall results to those reported by Medeiros et al. The inhibitors used by Trinchese et al were not quite as bioavailable—one was administered by intraperitoneal injection and the other was orally administered but required that the agent be solubilized in detergent, Tween 80. Although not quite as comprehensive in their analyses of their experimental animals, the investigators did not report any changes in Aβ levels despite the cognitive improvement observed. It is possible that the neuropathological and behavioral effects of calpain inhibition exhibit different sensitivities depending on the nature and extent of the enzyme inhibition. However, the apparent discrepancy in these results on Aβ homeostasis raises questions about the correlation of Aβ levels with cognitive changes, alerting us to the possibility that in the measurements of Aβ levels, the main neurotoxic species is not being specifically measured. In any event, as calpain activation is likely downstream of Aβ dysregulation, it may not be necessary to reduce Aβ concentrations, as long as the mechanism(s) of its neurotoxicity is substantially attenuated, as appears to be the case for both the Trinchese et al and the Medeiros et al studies. Given that each of the features of experimental Alzheimer's disease may evolve at different rates, further studies of dose and timing of these promising putative treatments are indicated.

Medeiros et al suggest that calpain inhibition may be not only neuroprotective, which it clearly is in this model, but also neurorestorative. However, this latter effect was not established by the evidence presented. To address this question, it would have been helpful for these authors to have examined transgenic animals before treatment was initiated to ascertain whether plaques and associated features already present regressed with calpain inhibitor treatment.

Conclusions

This study and its predecessors clearly highlight the attractiveness of calpain inhibition in the potential treatment of patients at risk for the development of Alzheimer's disease. For the identification of such patients, it would be most helpful to have specific and easily measured biomarkers indicative of early Alzheimer's disease. There has been much recent research activity on the utility of markers recognizable in the CSF of subjects at risk, as well as attempts to standardize their measurements across medical centers. At present, the combination of a decline of Aβ42 and an increment in total and phosphorylated tau (threonine 181) in the CSF along with MRI and amyloid imaging appear to represent the current "gold standard" for the diagnosis of Alzheimer's disease and for its distinction from other forms of mild cognitive impairment, including those associated with aging.[20] Perhaps to be added to these biomarkers relevant to the calpain network, is the presence of 150-kDa spectrin fragment resulting from calpain proteolysis in the CSF.[7] Recent studies on the reversible early dysregulation of olfactory function in a mouse model of β-amyloidosis may portend another valuable functional biomarker for use in evaluating potential therapies.[21,22] These biomarkers will be very helpful in the evaluation of prospective treatments, including calpain inhibition.

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References

1. Querfurth HW, LaFerla FM: Alzheimer's Disease. N Engl J Med 2010, 362:329–344
2. Medeiros R, Kitagawa M, Chabrier M, Cheng D, Baglietto-Vargas D, Kling A, Moeller A, Green KN, LaFerla FM: Calpain inhibitor A-705253 mitigates Alzheimer's disease like pathology and cognitive decline in aged 3xTgAD mice. Am J Pathol 2012; 181:616–625
3. Goll DE, Thompson VF, Li H, Wei W, Gong J: The Calpain System. Physiol Rev 2003, 83:731–801
4. Green KN, LaFerla FM: Linking calcium to Aβ and Alzheimer's disease. Neuron 2008, 59:190–194
5. Saito K, Elce JS, Hamos JE, Nixon RA: Widespread activation of calcium-activated neutral proteinase (calpain) in the brain in Alzheimer's disease: a potential molecular basis of neuronal degeneration. Proc Natl Acad Sci USA 1993, 90:2628–2632
6. Rao MV, Mohan PS, Peterhoff CM, Yang DS, Schmidt SD, Stavrides PH, Campbell J, Chen Y, Jiang Y, Paskevich PA, Cataldo AM, Haroutunian V, Nixon RA: Calpastatin depletion (CAST) in Alzheimer's disease accelerates cytoskeleton disruption and neurodegeneration: neuroprotection by CAST expression. J Neurosci 2008, 28:12241–12254
7. Higuchi M, Iwata N, Matsuba Y, Takamo J, Suemoto T, Maeda J, Ji B, Ono M, Staufenbiel M, Suhara T, Saido TC: Mechanistic involvement of the calpain-calpastatin system in Alzheimer's neuropathology. FASEB J 2012, 26:1204–1217
8. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM: Triple transgenic model of Alzheimer's disease with plaques and tangles; intracellular Aβ and synaptic dysfunction. Neuron 2003, 39:409–421
9. Lubisch W, Beckenbach E, Bopp S, Hoffman HP, Kartal A, Kastel C, Lindner T, Metz-Garrecht M, Reeb J, Regner F, Vierling M, Moller: Benzoylalanine-derived ketoamides carrying vinylbenzyl amino residues: discovery of potent water-soluble calpain inhibitors with oral bioavailability. J Med Chem 2003, 46:2402–2412
10. Sinjoanu RC, Kleinschmidt S, Bitner RS, Brioni JD, Moeller A, Ferreira A: The novel calpain inhibitor A 705253 potently inhibits oligomeric beta-amyloid-induced dynamin 1 and tau cleavage in hippocampal neurons. Neurochem Int 2008, 13:359–369
11. Granic I, Nyskas C, Luiten PG, Eisel UL, Halmy LG, Gross G, Schoemaker H, Moller A, Nimmrich V: Calpain inhibition prevents amyloid β-induced neurodegeneration and associated behavioral dysfunction in rats. Neuropharmacology 2010, 59:334–342

12. Nimmrich V, Reymann KG, Strassburger M, Schoder UH, Gross G, Hahn A, Schoemaker H, Wicke K, Moller A: Inhibition of calpain prevents NMDA-induced cell death and β-amyloid-induced synaptic dysfunction in hippocampal slice cultures. Br J Pharmacol 2010, 159:1523–1531
13. Ma W, Han W, Greer PA, Tuder RM, Toque HA, Wang KK, Caldwell RW Su Y: Calpain mediates pulmonary vascular remodeling in rodent models of pulmonary hypertension and its inhibition attenuates pathological features of disease. J Clin Invest 2011, 121:4548–4566
14. Johnson JD, Otani K, Bell GI, Polonsky KS: Impaired insulin secretion in transgenic mice over expressing calpastatin in pancreatic beta cells. Islets 2009, 1:242–248
15. Liang B, Duan BY, Zhou XP, Gong JX, Luo ZG: Calpain activation promotes BACE1 expression, amyloid precursor protein processing, and amyloid plaque formation in a transgenic mouse model of Alzheimer's disease. J Biol Chem 2010, 277:27737–27744
16. Wahrle SE, Jiang H, Parsadanian M, Kim J, Li A, Knoten A, Jain S, Hirsch-Reinshagen V, Wellington CL, Bales KR, Paul SM, Holtzman DM: Overexpression of ABCA1 reduces amyloid deposition in PDAPP mouse model of Alzheimer's disease. J Clin Invest 2008, 118:671–682
17. Martinez LO, Agerholm-Larsen B, Wang N, Chen W, Tall AR: Phosphorylation of a PEST sequence in ABCA1 promotes calpain degradation and is reversed by apo A-I. J Biol Chem 2003, 278: 37366–37374
18. Jiang Q, Lee CYD, Mandrekar S, Wilkinson B, Cramer P, Zelcer N, Mann K, Lamb B, Willson TM, Collins JL, Richardson TC, Smith JD, Comery TA, Riddell D, Holtzman DM, Tontonoz P Landreth GE: ApoE promotes the proteolytic degradation of Aβ. Neuron 2008, 58:681–693
19. Trinchese F, Fa M, Liu S, Zhang H, Hidalgo A, Schmidt SD, Yamaguchi H, Yoshii N, Mathews PM, Nixon RA, Arancio O: Inhibition of calpains improves memory and synaptic transmission in a mouse model of Alzheimer's disease. J Clin Invest 2008, 118:2796–2807
20. Shaw LM, Vanderstichele H, Knapik-Czajka M, Figurski M, Coart E, Biennow K, Soares H, Simon AJ, Leweczuk P, Dean RA, Siemers E, Potter W, Lee VM, Trojanowski JQ, Alzheimer's Disease Neuroimaging Initiative: Qualification of the analytical and clinical performance of CSF biomarker analyses in ADNI. Acta Neuropathol 2011;121:597–609
21. Wesson DW, Borkowski AH, Landreth GE, Nixon RA, Levy E, Wilson DA: Sensory network dysfunction, behavioral impairments and their reversibility in an Alzheimer's β-amyloidosis mouse model. J Neurosci 2011, 31:15962–15971
22. Wesson DW, Levy E, Nixon RA, Wilson DA: Olfactory dysfunction correlates with amyloid-β burden in an Alzheimer's disease mouse model. J Neurosci 2010, 30:505–514