Journal Club

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Aducanumab Therapy Ameliorates Calcium Overload in a Mouse Model of Alzheimer's Disease

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Review of Kastanenka et al.

The limited number of treatment options has made Alzheimer's disease (AD) a challenging problem for clinicians and patients. Currently, there are five Food and Drug Administration-approved drugs for the management of AD. Four of these drugs are acetylcholinesterase inhibitors and the other is memantine, an NMDA blocker designed to mitigate the potential effects of glutamate-induced calcium excitotoxicity (Parsons et al., 2013). Unfortunately, these drugs provide only temporary symptomatic relief and cannot halt or prevent disease progression (Parsons et al., 2013). Additional therapies devised to address the root biological mechanisms underlying AD pathogenesis would therefore be valuable.

According to the amyloid hypothesis, abnormal production, accumulation, or disposal of the amyloid-β (Aβ) protein is the primary driving force behind AD (Hardy and Selkoe, 2002). Therefore, research groups have exerted a great deal of effort to find ways to directly target Aβ levels in the brain. One approach has been to create anti-Aβ monoclonal antibodies to remove brain Aβ plaques. Promising results have emerged from mouse studies using transgenic AD lines showing reductions in Aβ plaque levels in the brain parenchyma. One such antibody, bapineuzumab, was shown to elicit Fc-mediated microglial phagocytosis of Aβ plaques (Panza et al., 2010). However, phase III clinical trials of both bapineuzumab and its sister drug solanezumab failed to show any overall clinical improvements in cognition or the disease itself (Doody et al., 2014; Salloway et al., 2014; Wisniewski and Goñi, 2015).

The most recently developed monoclonal antibody targeting Aβ is aducanumab by Biogen. It has been shown to bind aggregated Aβ, in the form of both soluble oligomers and insoluble fibrils, in the brain parenchyma. Studies in transgenic AD mouse lines showed that the antibody reduces plaque size in a dose-dependent manner. In addition to reductions in plaque size, the antibody slowed cognitive decline and was the first AD drug to show statistically significant reductions in plaque size in early human trials. Currently, aducanumab is in phase III clinical trials that are expected to be complete in 2022. There is a great deal of hope that aducanumab might be the first drug to combat the root causes of AD (Sevigny et al., 2016).

Evidence from experimental models and human subjects indicates that Aβ toxicity and disruption of neuronal calcium regulation are linked (LaFerla, 2002; Arundine and Tymianski, 2003; Mattson, 2007). Neurons use calcium signaling in numerous processes, and tight control of calcium levels is vital. Indeed, dysregulation of calcium homeostasis in neurons can be detrimental. Perturbations to intracellular calcium have been implicated in many neurodegenerative diseases including AD, Parkinson's disease, and Huntington's disease (Mattson, 2007; Berridge, 2014). Whether altered calcium homeostasis in AD precedes Aβ accumulation or vice versa is still controversial. It has been shown that Aβ-affected neurons have elevated calcium levels, disrupted calcium homeostasis, increased amounts of free and/or protein-bound calcium, and increased activity of calcium-dependent proteases (Mattson, 2007; Kuchibhotla et al., 2008). It has also been shown that $A\beta_{1-42}$ plaques aid in making calcium-permeable pores in cell membranes as well as in increasing the generation of reactive oxygen species, which also lead to elevated calcium levels (LaFerla, 2002). However, there is also evidence showing that calcium dyshomeostasis precedes Aβ function (Yoo et al., 2000). Therefore, studies on the link between calcium overload and Aβ function is useful in devising new therapies to treat AD.

A compelling study by Kastanenka et al. (2016) in *The Journal of Neuroscience* has examined the beneficial effects of aducanumab on clearing Aβ plaques and suggested that this drug might ameliorate calcium dysregulation in AD.

To assess the efficacy of aducanumab, the authors took an *in vivo* approach and directly applied a chimeric aducanumab an-

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alog ([ch]aducanumab) to the brain surfaces of 18-month-old mice from the well established Tg2576 AD model. Brain penetration of [ch]aducanumab and control antibodies was confirmed using Cy-3-tagged versions. Three weeks after a single topical application of [ch]aducanumab, there was a significant decrease in amyloid plaque size and overall amyloid burden (percentage of the area occupied by amyloid per image) and an increase in plaque clearance rate compared with controls. Interestingly, the authors noted that [ch]aducanumab did not affect the appearance of new plaques, suggesting that its effects are mediated primarily by clearing existing plaques and potentially soluble Aβ oligomers assessed by methoxy-XO4 labeling of plaques. These results are consistent with preclinical and clinical trials demonstrating that aducanumab can reduce the overall amyloid burden in both AD patients and a transgenic AD mouse model (Sevigny et al., 2016). Unlike topical administration, long-term systemic administration of [ch]aducanumab did not lead to changes in plaque size, plaque number, plaque clearance rate, or overall amyloid burden relative to a control antibody in 22-month-old mice. Evidence that aged mice demonstrating significant plaque density are resistant to antiamyloid treatment may explain these findings (DeMattos et al., 2012).

Kastanenka et al. (2016) next assessed the effect of aducanumab on calcium homeostasis. Using a calcium indicator probe, they measured intracellular calcium concentration in neurites of wild-type and Tg2576 mice, which had a substantially elevated amount of Aβ present. Treatment with [ch]aducanumab rescued the elevated calcium levels in transgenic neurites, restoring them to control levels within 2 weeks. At the end of the 6-month treatment period, there were no longer any neurites with elevated calcium levels. Furthermore, elevations in cell body calcium levels were also ameliorated with [ch]aducanumab treatment.

Calcium levels in neurons are rapidly detected by calcium sensor proteins, including visinin-like protein (VILIP), and plasma calcium levels are regulated by internal calcium stores, including the endoplasmic reticulum, which possesses store-operated calcium cannels to regulate both calcium entry [sarcoendoplasmic reticulum calcium ATPase (SERCA) pump] and calcium release through [inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$] receptors and ryanodine receptors (RyRs) (Mattson, 2007). Kastanenka et al. (2016) found that Tg2576 brains had increased VILIP expression compared with wild-type brains, and VILIP expression decreased upon treatment with [ch]aducanumab. In addition, expression of SERCA was reduced in Tg2576 cells under control conditions, and this was rescued by treatment with [ch]aducanumab. In contrast, neither the levels nor the number of cells expressing IP_3 receptors and RyRs changed in any groups of mice.

Aβ deposits promote NMDA receptor endocytosis, and, in mice bearing a familial Swedish mutation of APP, the number of surface NMDA receptors was decreased, indicating that Aβ can regulate the levels of NMDA receptors in neurons (Snyder et al., 2005). Although the overall levels NMDA receptor subunits NR1 and NR2 (specifically NR2A and NR2B) were similar in wild-type and transgenic animals in the study by Kastanenka et al. (2016), the number of cells expressing NR1, NR2A, and NR2B was lower in control-treated Tg2576 animals than in wild-type animals. Treatment with [ch]aducanumab restored the levels of NR1 and NR2A, but not that of NR2B.

In summary, Kastanenka et al. (2016) report that short-term application of a murine analog of aducanumab clears amyloid plaques and restores calcium homeostasis in affected neurons. The role of calcium in the pathogenesis of Alzheimer's disease is becoming elucidated via studies confirming alterations in calcium buffering in APP and presenilin1 mutants and rapid calcium elevations upon Aβ application in culture (Kuchibhotla et al., 2008). Therefore, ameliorating calcium dyshomeostasis with aducanumab might be of great therapeutic value in terms of preventing disease progression in early stages. The later stages of the disease and older Aβ plaques may be resistant to antiamyloid antibody therapy as the authors demonstrate with long-term administration of aducanumab in older animals.

The pathological form of Aβ in AD is still unclear. Several animal studies point to soluble oligomers as being the primary toxic culprit (Hillen et al., 2010). Moreover, several studies have shown that plaques, which were previously thought to be the deleterious species in AD, may act as a beneficial sink for the more harmful soluble forms (Cheng et al., 2007; Treusch et al., 2009). Thus, it follows that antibody therapies that are specific to soluble oligomeric Aβ might be effective. However, both bapineuzumab and solanezumab bind soluble monomeric and oligomeric Aβ. Much like aducanumab, bapineuzumab targets monomeric, oligomeric, as well as fibrillar Aβ (solanezumab shows little affinity for insoluble Aβ; Goure et al., 2014). Why did bapineuzumab and solanezumab fail in clinical trials? While there is no definitive answer to this question, significant amyloid load in the blood preventing the antibodies from reaching CNS targets in bapineuzumab trials and the use of non-AD participants in solanezumab trials may have caused these failures (Salloway et al., 2014; Abbot and Dolgin, 2016). Considering these previous efforts, the phase I trial for aducanumab shows promise, as the trials demonstrate a slowing of cognitive decline in patients prescreened with PET scanning to ensure amyloid pathology (Sevigny et al., 2016). Improvement in experimental design and significant cognitive benefits in an underpowered study provide hope that aducanumab may be successful in future trials.

As demonstrated by Kastanenka et al. (2016), there is also the possibility that the main benefits of aducanumab do not occur through Aβ reduction but through the correction of calcium homeostasis. Future behavioral experiments will need to address whether ameliorating calcium levels through aducanumab treatment in AD mouse models leads to cognitive improvements. Alzheimer's disease is a complicated disorder and, as several studies have shown, solely targeting Aβ might be a suboptimal treatment. A multivariate therapy such as aducanumab that addresses both Aβ load as well as downstream neurochemical effects is likely a necessity for the treatment of AD.

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