Alzheimer's Disease: Prevention and Therapy

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Posted on: July 7, 2017

Abstract

It has been well established that aerobic exercise can increase the production of brain-derived neurotrophic factor (BDNF), thus beneficial for the prevention of Alzheimer's disease (AD). In ApoE4 carriers, this capacity could be impaired. They may consider other approaches to increase BDNF such as intellectual activity, melatonin and lithium. On the basis of the BDNF Cascade Hypothesis, therapeutic development should target the steps before Ca^{2+} overload in the cascade, since the dramatically increased cytosolic Ca^{2+} may trigger a variety of damaging processes that would be very hard to inhibit. Aβ-targeting therapies are not recommended because Aβ production, and subsequent formation of Aβ oligomers and plaques, occur after Ca^{2+} overload. The most promising target could be the 4-repeat Tau, which plays a pivotal role in hyperexcitability (Paper 2) and consequently Ca^{2+} overload. On the other hand, the antibody against Tau's phosphatase-activating domain (PAD) is likely to alleviate disease progression as PAD exposure is critical for pathology spreading (Paper 10).

Introduction

In the past 25 years, therapeutic strategies for Alzheimer's disease (AD) have been based mainly on the Amyloid Cascade Hypothesis (Hardy and Higgins, 1992; Selkoe and Hardy, 2016) which asserts that the amyloid beta peptide (Aβ) is at the heart of AD. To date, none of the Aβ-targeting therapy produced any reduction in neurofibrillary pathology or improvement in cognitive performance of patients with AD (Dai et al., 2017). This has raised doubts on the validity of the Amyloid Cascade Hypothesis (Herrup, 2015). Accordingly, a growing number of therapeutic studies are focusing on another major player in AD: Tau protein.

The BDNF Cascade Hypothesis presented in previous papers centers on the Tau protein. On the basis of this hypothesis, Paper 10 suggests that the pathology spreading between neurons could arise from "**hyperexcitability transfer**" mediated by the exposure of phosphatase-activating domain (PAD) in pathological Tau proteins (phosphorylated and/or truncated). Thus antibodies against PAD should be able to produce beneficial results by neutralizing PAD exposure. However, the pathological Tau, and the consequent PAD exposure, occurs late in the BDNF cascade. The neuron could have been damaged before PAD exposure. This paper will discuss interventions on earlier steps, which may prevent the generation of pathological Tau in the first place.

BDNF Production

BDNF has been suggested for the treatment of AD and other neurological disorders (Nagahara and Tuszynski, 2011). However, therapeutic delivery of recombinant human BDNF to affected neurons faces several challenges, including short in vivo half-life, low permeability through the blood-brain barrier and high manufacturing costs (Géral et al., 2013). Instead of directly delivering BDNF to the brain, there are many approaches that can increase BDNF level. Some of them are presented below.

Physical Exercise

The easiest way to produce BDNF is **aerobic exercise** (e.g., jogging, rowing, swimming, or cycling), but not resistance exercise (e.g., weight lifting) (Dinoff et al., 2016). The aerobic exercise can elevate cerebral blood flow velocity (Ainslie et al., 2008), creating greater shear stress on the surface of blood vessels. This in turn stimulates the production of nitric oxide (NO) via activation of mechanosensors such as G-protein-coupled receptors, ion channels, PECAM-1 and VE-Cadherin (Lu and Kassab, 2011; Givens and Tzima, 2016). NO is a hydrophobic molecule, capable of passing through the cell membrane and diffusing to other cells. NO up-regulates BDNF in target cells (Chen et al., 2005), possibly via the NO/sGC/cGMP pathway (Chalimoniuk et al., 2015; Furini et al., 2010).

The resistance exercise also increases NO production, but only at high intensity. Light resistance exercises do not affect NO levels (Güzel et al.,

2007). At high intensity, the resistance exercise may trigger significant inflammatory responses, such as elevation of interleukin-6, which could reduce the BDNF level (Verbickas et al., 2017), thus negating the up-regulation by NO.

Recently, it was reported that the process of aerobic exercise leading to BDNF production is impaired in elderly African Americans with ApoE4 (Allard et al., 2017). This report has very important implications as ApoE4 is a well-documented risk factor for AD (Paper 9). In Northern Europe, the prevalence of homozygote ApoE4 carriers is as high as 14%, and 60% of AD patients carry homozygote ApoE4 (Ward et al., 2012). The high incidence of AD in ApoE4 carriers could be related to the deficit of exercise-induced BDNF production. If this is confirmed by future studies, they should consider other approaches to produce BDNF.

Intellectual Activity

After BDNF is produced in the cell body, they are packaged into vesicles and transported to presynapses, not postsynapses (Andreska et al., 2014). Like other neurotransmitters, the release of BDNF from presynapses depends on neuronal activity (Park et al., 2014). Therefore, intellectual activity should be able to stimulate BDNF release into the synaptic cleft. The released BDNF then binds with TrkB located on both presynaptic and postsynaptic membranes, triggering its own production in both presynaptic and postsynaptic neurons (Paper 4). This explains why playing GO game ameliorates AD manifestations by up-regulating BDNF levels (Lin et al., 2015). In accord with the BDNF Cascade Hypothesis, another study found that longer education reduces total and phosphorylated Tau (Almeida et al., 2015).

Melatonin

Melatonin is a powerful antioxidant. It can also exert beneficial effects by acting on its receptors: MT1 and MT2 (Jenwitheesuk et al., 2014). Melatonin has been demonstrated to increase BDNF production (Imbesi et al., 2008; Zhang et al., 2013; Rudnitskaya et al., 2015) through its receptors, rather than its antioxidant property (Imbesi et al., 2008). The novel antidepressant, agomelatine, acts on both melatonin and 5-HT2C receptors. Administration

of agomelatine also significantly increases the BDNF level (Soumier et al., 2009; Calabrese et al., 2011; Gumuslu et al., 2014; Martin et al., 2017).

Lithium

Lithium is widely prescribed for the treatment of bipolar disorder. Its beneficial effects may involve several different mechanisms (Dell'Osso et al., 2016). Among them, lithium is known to up-regulate BDNF level (Hashimoto et al., 2002; De-Paula et al., 2016). Bipolar patients treated with lithium have reduced risk for AD, suggesting that lithium could be a therapeutic for AD (Lauterbach et al., 2016). Chronic treatment with microdose lithium did produce positive results by elevating BDNF level (Nunes et al., 2013; Nunes et al., 2015). Lithium is beneficial not only for AD, but also for other neurodegenerative disorders if treatment started at the earlier stages of the disease (Lauterbach, 2016; Dell'Osso et al., 2016). These findings agree with the BDNF Cascade Hypothesis for neurodegeneration (Paper 4 and Paper 5).

Timing is important for the success of lithium treatment at regular doses. Starting at a late stage of AD would not produce beneficial effects (Fiorentini et al., 2010; Lauterbach et al., 2016). This could be due to the involvement of GSK-3 activation, as discussed in the next section.

GSK-3 Inhibitors

Tau phosphorylation plays a critical role in AD. GSK-3 has the capacity to phosphorylate more sites than other Tau kinases such as Cdk5, CaMKII and PKA (Wang et al., 2007). Phosphorylation of its target sites may reduce microtubule binding (Thr231), sequester normal Tau from microtubules (AT8 epitope: Ser199/Ser202/Thr205), and promote self-aggregation of Tau into filaments (Ser396, Ser404 and Ser422) (Gong and Iqbal, 2008). These findings suggested that Tau hyperphosphorylation might lead to AD and the GSK-3 inhibitor should be able to halt the disease progression. Unfortunately, therapeutic development along this line was disappointing. A series of highly selective and potent GSK-3 inhibitors have failed in pre-clinical assessment. (Cormier and Woodgett, 2017). Tideglusib is the only GSK-3 inhibitor that reached phase II clinical trials for the treatment of

AD, but produced no clinical benefit for patients with mild to moderate AD (Lovestone et al., 2015).

The degree of Tau phosphorylation increases as the disease advances. In neurofibrillary tangles (NFTs), Tau is hyperphosphorylated. Recently, researchers began to realize that Tau oligomers, not NFTs, are the true toxic species (Shafiei et al., 2017). At a more fundamental level, the toxicity could arise from PAD exposure (Paper 10). In a normal free Tau, PAD is hidden in the paper-clip conformation. Phosphorylation at the AT8 epitope cause PAD to expose, thereby triggering toxic processes. However, further phosphorylation at Ser396 and Ser404 (GSK-3 targets) leads to compaction of the paper-clip (Jeganathan et al., 2008), which is expected to reduce PAD exposure and thus ameliorating toxicity. This observation is consistent with the findings that phosphorylation at Ser396 and Ser404 promotes the formation of NFTs (Abraha et al., 2000) where PAD is not exposed (Combs et al., 2016). In addition, phosphorylation at Ser422 (a GSK-3 target) prevents Tau cleavage by caspase 3 (Guillozet-Bongaarts et al., 2006). Tau hyperphosphorylation at the axon initial segment has been shown to attenuate excitability (Paper 2). Therefore, while the early phosphorylation at the AT8 epitope is harmful, further phosphorylation by GSK-3 could be neuroprotective. This may explain why GSK-3 inhibitors did not produced clinical benefit for patients with mild to moderate AD.

Lithium is a well-documented GSK-3 inhibitor. At microdose, it can elevate BDNF level, but insufficient to affect GSK-3 activity (Nunes et al., 2015). At regular doses, it may reduce AD risk by elevating BDNF as well as inhibiting GSK-3. However, like other GSK-3 inhibitors, lithium is not effective for mild to moderate AD.

Generally speaking, intervention at the step after Ca^{2+} overload is not a good approach. Once the cytosolic Ca^{2+} is dramatically increased, it may trigger a variety of damaging processes that would be very hard to inhibit. In retrospect, the failure of Aβ-targeting therapies is expected since Aβ production, and subsequent formation of Aβ oligomers and plaques, occur after Ca^{2+} overload (Figure 1). Likewise, calpain inhibitors are unlikely to succeed either. ABT-957 is a calpain inhibitor that entered phase 1 clinical trial in August 2014, but was terminated in June 2016 due to insufficient target engagement (Web Link).

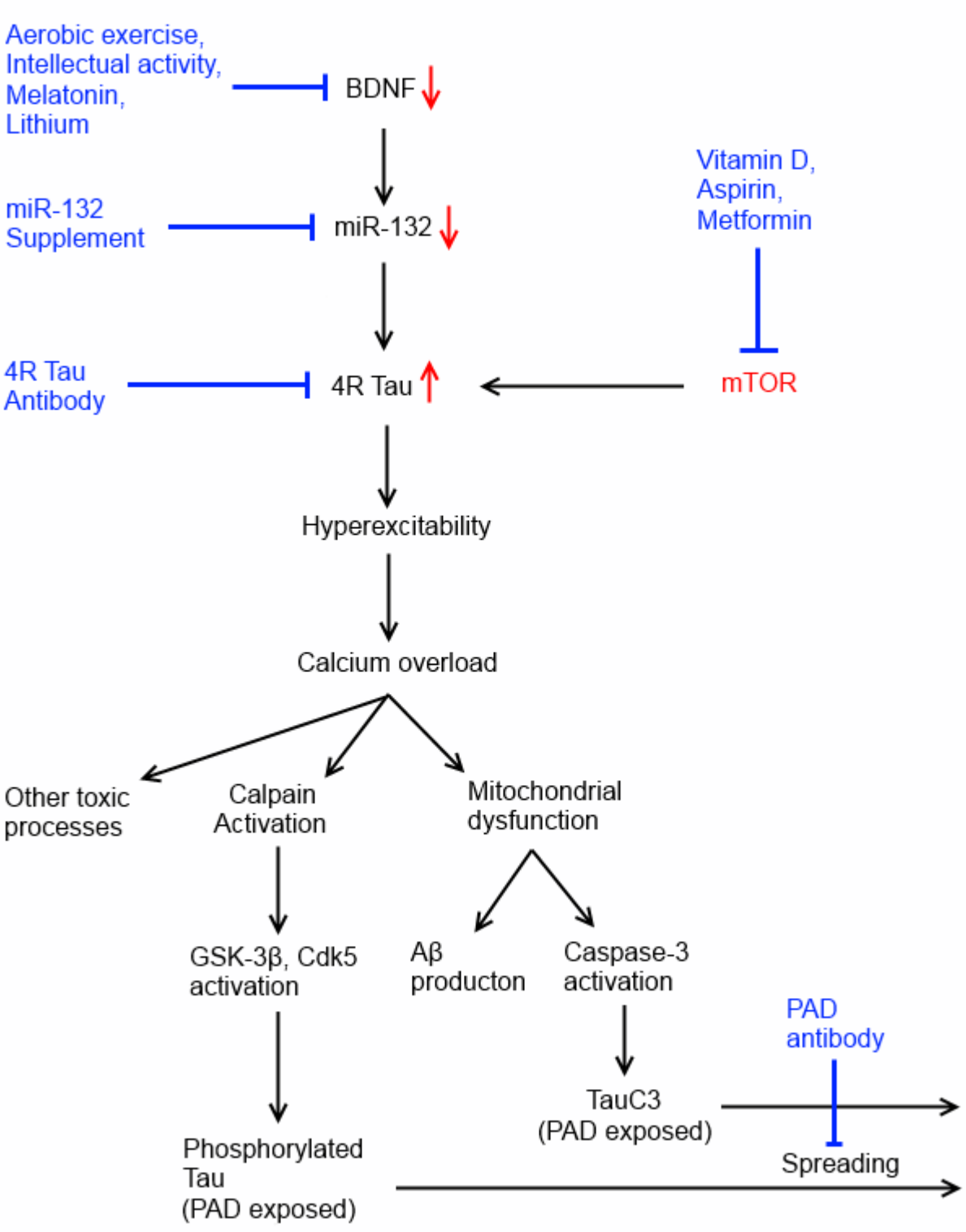

Figure 1. Recommended interventions (in blue color) on the BDNF cascade in Alzheimer's disease. Once the cytosolic Ca^{2+} is dramatically increased, it may trigger a variety of damaging processes that would be very hard to inhibit. Therefore, prevention and therapy should target the steps before Ca^{2+} overload in the cascade.

The Achilles Heel of AD

According to the BDNF Cascade Hypothesis, AD is fundamentally caused by Ca^{2+} overload, which in turn arises from hyperexcitability. Several lines of evidence suggest that elevated 4-repeat (4R) Tau is the major cause of hyperexcitability and ensuing Tau pathology.

1. Elevated 4R-Tau in a human-Tau mouse model induced more severe seizures at early time points, driving Tau phosphorylation and aggregation (Schoch et al., 2016).
2. The dramatically elevated 4R Tau is crucial for the novel modeling system (called "3D cell culture") to recapitulate AD features (D'Avanzo et al., 2015).
3. Introduction of wild-type 4R Tau into wild-type animals triggered caspase-3 activation, Tau truncation and Tau aggregation (de Calignon et al., 2010).
4. Microinjection of human 4R Tau into the presynapse of squid neuron triggered Ca^{2+} overload, GSK-3 and Cdk5 activation, Tau phosphorylation at the AT8 epitope, PAD exposure and presynaptic dysfunction (Moreno et al., 2016).

Therefore, 4R Tau could be the "Achilles heel" of AD. By reducing 4R Tau, all subsequent pathological events in the cascade would be inhibited. This could be achieved by using 4R Tau antibody, which is commercially available. However, the antibody may not be able to reach its target inside the neuron. The technology to overcome this difficulty is an area of intensive research (Marschall and Dübel, 2016). Before the 4R Tau antibody can be successfully delivered to its intracellular target, we may resolve to indirect approaches such as BDNF production (discussed above), mTOR inhibition and miR-132 supplement.

mTOR Inhibitors

mTOR is the risk factor for most human diseases including AD (see "mTOR, the Ultimate Risk Factor"). Its activation promotes Tau production (Caccamo et al., 2013; Tang et al., 2013; Tang et al., 2015). Therefore,

inhibiting mTOR should be able to reduce the AD risk. The following is a list of mTOR inhibitors that do not need prescription.

- Vitamin D (Lisse and Hewison, 2011).
- Aspirin (Din et al., 2012).
- Polyphenols (in coffee) (Pietrocola et al., 2014).
- Oleocanthal (in olive oil) (Khanfar et al., 2015).

The commonly prescribed anti-diabetic drug, metformin, can also inhibit mTOR activity (Gong et al., 2014).

miR-132 Supplement

miR-132 is one of many microRNAs that have the capacity to repress translation of their target mRNA (Paper 4). A growing body of evidence suggests that BDNF exerts its beneficial effects via up-regulation of miR-132 (Numakawa et al., 2011; Zheng et al., 2013; Marler et al., 2014), which targets the mRNA of both Tau protein and a splicing factor, polypyrimidine tract-binding protein 2 (PTBP2). Thus, miR-132 regulates not only the total Tau level, but also the ratio between 4R and 3R Tau. miR-132 deficiency has been shown to cause elevated total Tau and higher 4R:3R Tau ratio (Smith et al., 2011; Smith et al., 2015). Therefore, miR-132 supplement should be a promising therapeutic strategy. However, delivery of microRNAs is a challenging task because they could be degraded before reaching the target cell. Recently, exosomes have emerged as a powerful drug delivery system (Alvarez-Erviti et al., 2011; Kalani et al., 2014; Mathiyalagan and Sahoo, 2017). Exosomes are the smallest naturally-occurring membranous vesicles, involved in cell-to-cell communication (Cervio et al., 2015). They may carry a variety of proteins, lipids, non-coding RNAs, mRNA, and microRNA. For drug delivery, their membrane can be modified to enhance tissue-specific targeting. Since exosomes are produced in natural biological systems, immunogenicity is low. Furthermore, exosomes can cross the blood-brain barrier (El Andaloussi et al., 2013).

The Bull's-eye of Pathology Spreading

Tau aggregates were thought to be the toxic substance that mediates pathology spreading in AD. Tau aggregation inhibitors were developed on the basis of this assumption. One of them, leuco-methylthioninium bis (LMTM), has managed to enter phase 3 clinical trial, but "the results do not suggest benefit of LMTM as an add-on treatment for patients with mild to moderate Alzheimer's disease" (Gauthier et al., 2016).

Recently, the PAD of Tau has emerged as a central player in pathology spreading. Its underlying mechanism is consistent with the BDNF Cascade Hypothesis (Paper 10). Although PAD antibody is too late to inhibit some pathological processes in the originally affected neuron (e.g., in entorhinal cortex), this approach could be effective in alleviating pathology spreading to connected neurons (e.g., in hippocampus). Indeed, PAD antibody has been shown to exert positive effects in a cellular model (Agadjanyan et al., 2017). The results in an animal model are even more encouraging. Not only can PAD antibody suppress Tau hyperphosphorylation in the hippocampus, it also reduces Aβ level in the forebrain and amyloid plaques in subiculum (Dai et al, 2017).